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Research article

Herbal Gel for the Treatment of Arthritis: Formulation, Evaluation, and Standardization

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ABSTRACT

Introduction and Background: Arthritis patients now have another option in the form of the Siddha and Ayurvedic therapy systems. Cardiospermum halicacabum and Vitex negundo are the two herbs most frequently used in conventional medicine for the treatment of arthritis.

Material and Methods: The mature fresh leaves of Vitex negundo and Cardiospermum halicacabum were procured from Palakkad, Kerala. The following substances were procured from Sigma-Aldrich USA: Freund's complete adjuvant (FCA), diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate. Carbopol 934 and Carbopol 940 were procured from Loba Chemie Pvt. Ltd. in Mumbai.

Results: Physical appearance, net content, viscosity, extrudability, pH, spreadability, in vitro diffusion profile, and primary skin irritation tests were conducted on a set of 12 herbal gel formulations made with 1.5% of gelling agents carbopol 934 (F1-F6) and carbopol 940 (F6-F12). Anti-arthritic activity was measured using the Freund's Complete Adjuvant induced arthritis method, and the stability of the topical herbal gel formulation was studied in accordance with ICH recommendations. Histopathological analysis, In vitro detection of blood biomarkers, and

measurements of body weight and paw volume were also performed. Gel formulations were consistent, stable, and safe to use. The release characteristics of formulation F5 were the most favorable compared to the others.

Conclusion: An effective topical herbal gel for the treatment of arthritis was found to be the F5 formulation, which combines 2% CHME and 2% VNME with 1.5% carbopol 934. More clinical trials are needed to determine how effective this formulation is in treating inflammatory illnesses of the joints.

Keywords: Medicinal plants, Vitex negundo, Cardiospermum halicacabum, and arthritis

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INTRODUCTION

Arthritis is a prevalent autoimmune condition that impacts approximately 0.5- 1% of the global population. The pharmaceutical treatments frequently provided for Rheumatoid Arthritis encompass steroidal, non-steroidal anti-inflammatory, disease modifying antirheumatic, and immunosuppressant agents [1, 2]. These medications have been seen to elicit diverse adverse effects, such as gastrointestinal disorders, immunodeficiency, and humoral changes. The Siddha and Ayurvedic systems of treatment are gaining recognition as viable alternatives for the treatment of arthritis. Cardiospermum halicacabum and Vitex negundo are the two plant species that are frequently employed in traditional medicinal practices for the purpose of treating arthritis [3, 4]. Cardiospermum halicacabum (CH), a member of the Sapindaceae family, has a long history of utilization in traditional Chinese medicine for its therapeutic properties in addressing inflammation, rheumatism, and different ailments. The ethanol extract of CH has been found to exhibit anti-inflammatory properties by inhibiting the production of COX-2, TNF-α, and iNOS in RAW264.7 cells when stimulated by LPS [5-7].

Empirical pharmacological investigations have demonstrated the presence of analgesic and vasodepressant properties, as well as anti-pyretic effects against yeast-induced pyrexia in rats. Additionally, these studies have revealed anti-malarial and anti-oxidant activities, along with the ability to mitigate ethanol-induced gastric ulceration in rats. Furthermore, the tested compound has been observed to suppress the production of TNF-α and nitric oxide in human peripheral blood mononuclear cells [8, 9].

In addition, they are utilized for their tonic properties, as well as their ability to operate as vermifuges, lactagogues, emmenagogues, antibacterial agents, antipyretics, and anti-histaminic agents [10]. Currently, there is a lack of research on the preclinical investigations of CH and VN in relation to their anti-arthritic activity when used in topical gel form. In order to address this gap, a topical herbal gel was formulated using CH and VN, and afterwards assessed for its potential anti-arthritic effects. The objective of this study was to provide scientific evidence supporting the utilization of these plants in the treatment of arthritis [11].

The primary purpose and objectives of this study were to develop, assess, and establish standardized parameters for a herbal gel intended for the management of arthritis. Herbal medicines continue to serve as the predominant form of primary health care for around 75-80% of the global population, particularly in developing nations. This preference is mostly attributed to their greater cultural acceptance, enhanced compatibility with the human body, and less incidence of adverse effects. Herbal medications encompass the utilization of various plants or their components for the purpose of addressing injuries, diseases, or illnesses. These remedies are employed both as preventive measures and as therapeutic interventions, aiming to mitigate maladies and foster overall well-being and recuperation [13, 14].

MATERIALS AND METHODS

Materials

The fully developed foliage of Vitex negundo and Cardiospermum halicacabum was procured from Palakkad, Kerala. The following substances were procured from Sigma-Aldrich USA: Freund's complete adjuvant (FCA), diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate. Carbopol 934 and Carbopol 940 were procured from Loba Chemie Pvt. Ltd. located in Mumbai.

Extract Preparation

Careful processing was performed on the leaves of Vitex negundo and Cardiospermum halicacabum to eliminate any remaining earthy debris and residual materials before they were cleaned and shade dried. The leaves of Cardiospermum halicacabum were ground into a powder and then extracted with methanol in a Soxhlet apparatus for 72 hours. Vitex negundo leaves were ground into a powder and then cold macerated in methanol for 7 days. After heating to 40 degrees Celsius in an IKA Rotary evaporator (Model No. RN 10 digital V, ILMAC Germany), both extracts were filtered, concentrated under decreased pressure, and then chilled to between 4 and 8 degrees Celsius [15].

Animals used

Strains developed from Wistar rats Male and female rats weighing between 150 and 200 g were chosen from the SRM Institute of Science and Technology, Tamil Nadu, India to test anti-arthritic effects. For the acute toxicity investigation, we employed albino female mice weighing 20-30 g, and for the primary skin irritation test, we used albino rabbits. They were subjected to strict environmental controls such as a 10-14 hour light/dark cycle, 50-50% relative humidity, and a temperature of 23.2 degrees Celsius. The animals had ad libitum access to food and drink and were housed in polypropylene cages with sterile rice husk bedding. All procedures involving animals were authorized by the Institutional Animal Care and Use Committee and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA) [16].

Gel base Preparation

To prevent clumping, 60 mL of Carbopol 934 was dissolved gently with stirring in 1 h of demineralized water. Then, in separate vials of 10 mL of demineralized water, disodium edetate and triethanolamine were dissolved and agitated for 10 minutes. For 10 minutes, while swirling constantly, I combined 4.83 mL of propylene glycol with 12 mL of distilled water. After adding disodium edetate and triethanolamine solution to the carbopol solution and stirring for 10 minutes, the pH was corrected to 7.4. Then, after swirling for 10 minutes, propylene glycol solution was added to create a clear, consistent gel basis [17].

Gel formulation preparation

Gel formulations F1–F6 were made with a carbopol 934 gel base, while gel formulations F7–F12 were made with a carbopol 940 gel base, all in accordance with the drug formulation handbook. CHME (methanol leaf extract of Cardiospermum halicacabum). Table 1 lists the individual components of each formulation. Because of its higher quality, the F5 formulation made with carbopol 934 was tested for its anti-arthritic effectiveness [17, 18].

Table 1: Carbopol 934 and 940-containing gel formulations

Gel code	*Cardiospermum halicacabum extract (g)*	*Vitex negundo extract (g)*	Carbopol 934 (g)	Carbopol 940 (g)	Triethanol amine (g)	Disod. EDTA (g)	Prop Glycol (g)	water (100 g)
F1	0.6	0.6	1.4	NA	1.6	.006	6	
F2	1.0	1.0	1.4	NA	1.6	.006	6	

F3	1.4	1.4	1.4	NA	1.6	.006	6	Quantity Sufficient
F4	2.1	2.0	1.4	NA	1.6	.006	6	
F5	2.4	2.6	1.4	NA	1.6	.006	6	
F6	3.0	3.0	1.4	NA	1.6	.006	6	
F7	0.4	0.7	NA	1.6	1.6	.006	6	
F8	1.1	1.0	NA	1.6	1.6	.006	6	
F9	1.4	1.4	NA	1.6	1.6	.006	6	
F10	2.1	2.0	NA	1.6	1.6	.006	6	

Topical herbal gel formulation Quality control Study

Estimation of active constituents in gel formulation

Each formulation (1 g) was placed in a 50 mL volumetric flask and diluted with methanol to reach the desired volume. The flask was then vigorously shaken to ensure complete dissolution of the active components in methanol. The solution underwent filtration using Whatman filter paper, following which a volume of 0.1 mL of the resulting filtrate was extracted using a pipette and subsequently diluted to a final volume of 10 mL using methanol. The quantification of active components was performed using spectro photometric analysis, with a reference curve constructed at a wavelength of 275 nm [19, 20].

Extrudability Test

A sealed collapsible tube, holding approximately 20 grams of gel, was compressed hard at the crimped end and secured with a clamp to prevent any retraction. The cap was detached and the gel was expelled. The quantity of the extruded gel was gathered and measured. The percentage of the gel that was extruded was determined [21, 22].

pH measurement

The pH of the gel was determined using a digital pH meter. The glass electrode was fully immersed in the gel system to ensure total coverage of the electrode. The experiment was conducted in triplicate, and the mean value of the three measurements was documented [22, 23].

Appearance and Homogeneity Study

The visual perception method was used to assess the physical appearance and uniformity of the manufactured gels.

Viscosity Test

The viscosity of the gel was measured at a temperature of 25°C using a Brookfield viscometer, with the viscometer's spindle rotating at a speed of 12 revolutions per minute.

Spreadability Test

Two sets of glass slides with standardized dimensions were obtained. The herbal gel mixture was applied onto one of the slides. The second slide was positioned above the gel, resulting in the gel being enclosed between the two slides within a spatial extent of 7.5 cm along the slides. A gel weighing one hundred grams was positioned on the upper slides, resulting in the gel being compressed evenly between the two slides to create a thin layer. The calculation of spreadability was performed utilizing the subsequent formula [24, 25]:

$$S = m \times l/t$$

Study of Anti-arthritic effect

The study investigated the effectiveness of a topical herbal gel formulation in treating arthritis produced by FCA in rats, using a method of topical treatment. The rats were partitioned into four cohorts, with each cohort including six specimens. Group 1 was subjected to topical application using a gel basis, which served as the typical control. Group 2 to 4 rats were experimentally induced with arthritis through the administration of a 0.1 mL (0.1% w/v) suspension of inactivated Mycobacterium TB bacteria, which had been homogenized in liquid

paraffin. This suspension was injected into the subplantar region of the left hind foot of the rats. Group 2 was designated as the control group for arthritis. Groups 2 to 4 were subjected to the administration of Freund's Complete Adjuvant (FCA) and afterwards observed for a period of 21 days to monitor the development of arthritis. Throughout the duration of the trial, the body weight and paw volume of both the control and treatment groups were assessed on the 4th, 8th, 14th, and 21st day using a digital Vernier caliper [24, 25].

Hematological Study

The animals who had undergone an overnight fast were administered anesthesia using ketamine. Blood samples were then obtained from the retro-orbital sinus and subsequently centrifuged at a speed of 10,000 revolutions per minute for a duration of 10 minutes. These blood samples were then analyzed to determine various hematological parameters. The hematological parameters, including the count of red blood cells, white blood cells (WBCs), the value of hemoglobin (Hb), and the erythrocyte sedimentation rate (ESR), were assessed using standard laboratory techniques [26].

Rat Skin irritation Test

Three young rabbits were accommodated in metallic enclosures equipped with perforated flooring. Water and a regular diet for rabbits were provided without restriction. The ambient temperature of the room was consistently regulated at 22 ± 3 °C, while the relative humidity ranged between 30% and 70%. The light conditions were manipulated to provide a consistent 12-hour period of artificial lighting on a daily basis. Twenty-four hours before to the test, the application of a dose was carried out, involving the removal of hair on the back and flanks of each rabbit. This process was performed meticulously, resulting in the exposure of an approximate area of 6 cm2 of skin. The gel formulation was uniformly administered onto a 4 cm2 region of the carefully trimmed skin of every rabbit. The subjective assessment and scoring of skin reactions at the application site were conducted on a daily basis at certain time points: 1 hour, 24 hours, 48 hours, 72 hours, 7 days, and 10 days [27, 28].

RESULTS AND DISCUSSION

In the realm of topical semisolid preparations, gel formulation is commonly favored due to several advantageous properties. These include a prolonged residence time on the skin, a high viscosity, the ability to moisturize flaky skin through occlusive properties, enhanced bioadhesiveness, reduced irritation, independence from the water solubility of the active ingredient, ease of application, and improved release characteristics. Numerous research have provided evidence suggesting that certain flavonoids found in herbs, namely luteolin and apigenin, exhibit properties that can effectively mitigate inflammation and alleviate symptoms associated with arthritis. Moreover, it has been found that the polyphenolic flavonoids apigenin and luteolin have the ability to permeate the human skin. As a result, a topical herbal gel formulation was developed to incorporate these flavonoids for the purpose of treating arthritis.

Topical herbal gel formulation and evaluation

The methanol extracts of Cardiospermum halicacabum and Vitex negundo were used to create a total of 12 gel formulations, each of which contained either 1.5% Carbopol 934 or Carbopol 940 polymer. Carbopol 934 and carbopol 940 were utilized as a gelling agent because of their many positive attributes (biodegradability, bioadhesion, biocompatibility, lack of irritation, and non-absorption). We found that of the two polymers employed, carbopol 934 had a greater gelling property than carbopol 940. The gel formulation using Carbopol 934 polymer showed promise as a carrier for regulated release of active phytoconstituents.

Quality control testing showed that gel formulations made using Carbopol 934 (F1 to F6) as the gelling agent were superior to those made with Carbopol 940, with the exception of spreadability characteristics, for which Carbopol 940 was good. Therefore, only the best herbal gel formulation, F5, underwent in vitro release and stability investigations. In vitro

diffusion studies were conducted on the six herbal topical gel formulations (F1 to F6) prepared with carbopol 934.

Herbal gel Quality control test

Carbopol polymers were used to create a total of twelve different gel formulations (F1–F12), which were then tested for their visual appeal, pH, viscosity, spreadability, net content, extrudability, and in vitro diffusion profile. The study's findings fell within the range recommended by the ICH guidelines, as shown in Table II. It was observed that the prepared gels were uniform in look and consistency. All formulations had pH values within a safe range for human skin, which is between 7.42 to 7.88, and hence did not irritate the skin.

The topical formulations were formulated with polymers for rapid drug release and to achieve and maintain a therapeutically effective drug concentration. There was no change in viscosity amongst the different gel formulations since the polymer concentration was kept constant at 1.5%. It has also been observed that a viscosity value of 0.38 to 0.39 poise is optimal for topical gel formulations created with carbopol polymers.

Table 2: Evaluation criteria for a 1.5% Carbopol 934-based topical herbal gel formulation

Code	Conc. (%)	pH*	Viscosity	Spreadability	Net content	Extrudability
F1	0.6	7.49	0.3714	31.20	99.4	Good
F 2	1.1	7.60	0.3784	44.00	104	Excellent
F 3	1.6	7.80	0.3713	55.40	106	Good
F 4	2.1	7.59	0.3781	65.10	104	Excellent
F 5	2.6	7.90	0.3790	70.40	100	Excellent

***In-vitro* diffusion profile Study**

Figure 1 shows the in vitro diffusion profile for each of the six different formulations (F1–F6). In vitro release experiments of the gel formulations were conducted in phosphate buffer saline (pH 7.4) because the pH of the membrane utilized was in the range of 5 to 7.8. All six formulations containing carbopol 934 showed nearly 100% release from the formulation within 5 h, as measured by in vitro release profiles. The produced topical herbal gel formulations showed very promising in vitro release characteristics, correlating well with commercially available diclofenac gel. Formulation F5 had the highest release percentage (98.4%) compared to formulas F1, F2, F3, F5, and F6.

Rat Skin irritation Study

The skin irritating impact of the prepared herbal gel was assessed, and it was noticed that none of the formulations caused erythema or edema, as indicated in Table 3. This lack of adverse effects persisted even after a 10-day research period, suggesting that the prepared herbal gel formulation can be considered safe.

Table 3: Herbal gel formulation 940 and 934 primary skin irritation test

Rabbit (No.)				Rabbit		Combined index
	I	II	III	Control	Avg.	
1 h						
Score (Erythema)	0	0	0	0	0.0	0.0

Score (Edema)	0	0	0	0	0.0	
24 h						
Score (Erythema)	0	0	0	0	0.0	0.0
Score (Edema)	0	0	0	0	0.0	
48 h						
Score (Erythema)	0	0	0	0	0.0	0.0
Score (Edema)	0	0	0	0	0.0	
72 h						
Score (Erythema)	0	0	0	0	0.0	0.0
Score (Edema)	0	0	0	0	0.0	
7 days						
Score (Erythema)	0	0	0	0	0.0	0.0
Score (Edema)		0	0	0	0.0	0.0
10 days						
Score (Erythema)	0	0	0	0	0.0	0.0
Score (Edema)	0	0	0	0.00		

Rat Paw volume Test

The rat paw volume changes were measured on the 25th, 29th, 35th, and 42nd days after the application of diclofenac sodium gel and herbal gel formulation (F5) via topical administration (Figure 5). The control groups with arthritis exhibited indications of arthritis progression, as evidenced by an increase in paw volume. A notable decrease in the volume of rat paws was seen in groups treated with diclofenac sodium gel and topical herbal gel formulation F5, 21 days following the introduction of FCA.

The results presented in Table VIII demonstrate that the test scores for arthritis, specifically induced by FCA, exhibited a significant decrease in both the group treated with diclofenac sodium gel and the group treated with the topical herbal gel formulation F5. This suggests that these treatments effectively reduced the pain associated with FCA-induced arthritis. A notable modification in the flexion pain test score, mobility score, and stance score was seen in all the experimental group of rats in comparison to the arthritic control rats. The modification of arthritic test scores provides evidence in favor of the anti-arthritic efficacy of the topical herbal gel formulation F5.

Out of the formulation the F5 formulation was chosen for an anti-arthritic study due to its positive results in quality control evaluation. The in vitro release characteristics of the F5 topical gel formulation were found to be promising and comparable to a commercially available diclofenac sodium gel.

Table 4: Diclofenac sodium, F5 herbal gel formulation and body weight alterations

Groups	body weight (g) (Initial)	Body wt. after 21 days of FCA induction	Body wt. after treatment 25th day	Body wt. after treatment 29th day	Body wt. after treatment 35th day	Body wt. after treatment 42nd day	Weight gain (g)
Control (Normal)	157.2 ± 0.47	170.3± 2.60	170.3 ± 2.20	180.00 ± 2.00	180.40 ± 2.30	190.60 ± 2.12	21.60±2.01
Control (Arthritic)	150.1 ± 0.24	150.20 ± 0.80	150.30 ± 0.80	150.10 ± 2.02	140.01 ± 2.20	140.20 ± 2.36	-10.02±2.34

Di. sodium topical gel	150.00± 2.45	150.10 ± 2.30	150.01± 2.50	150.30 ± 2.40	150.20 ± 2.35	140.00 ± 2.13	8.22±0.37
Topical herbal gel	150.6 ± 2.00	150.60 ± 2.20	150.10 ± 2.10	150.20 ± 2.40	150.00 ± 2.36	150.14 ± 2.63	6.12±0.54

Table 5: Efficacy of herbal gel formulation F5 against FCA-induced arthritis in rats

Groups	**Paw Volume of Rat (mm)**					
	Treatment (Before)		**Treatment (After)**			
	Initial	**After 21 days**	**25th day**	**29th day**	**35th day**	**42nd day**
Control (Normal)	5.12± 0.15	6.24± 0.34	6.11 ± 0.19	6.40±0.21	6.00 ± 0.14	6.80± 0.28
Control (Arthritic)	5.11± 0.13	11.47± 0.14	11.66 ± 0.32	11.70 ±0.21	11.80 ± 0.19	11.80 ± 0.40
Diclo. sodium gel	6.24± 0.14	11.11± 0.16	11.50 ± 0.18	10.71 ± 0.20	9.80 ± 0.31	9.31 ± 0.14
Topical herbal gel F5	5.86± 0.12	11.40± 0.17	11.41 ± 0.20	10.80 ± 0.20	10.08 ± 0.21	9.81 ± 0.37

Histopathological Study

The histological analysis of a healthy joint specimen revealed the presence of a normal joint space, intact surrounding soft tissue, synovium, and cartilage. The joint samples used for arthritis control had a pronounced inflammatory response in the surrounding soft tissue. The specimens from the groups treated with diclofenac sodium topical gel exhibited typical characteristics of cartilage, cortex, and marrow. The histopathological analysis of arthritic rats treated with topical diclofenac sodium gel and topical herbal gel formulation F5 revealed a notable decrease in inflammation inside the soft tissue around the joint, as observed in Figure 3 A and B, in comparison to the arthritic control rats.

CONCLUSION

The potential anti-arthritic effects of the topical herbal gel formulation that was developed could be attributed to the presence of luteolin and apigenin in the methanol leaf extracts of Cardiospermum halicacabum and Vitex negundo. The formulated product F5, which contains a combination of 2% CHME and 2% VNME along with 1.5% carbopol 934, exhibited potential as a topical herbal gel for the management of arthritis. Additional clinical investigations have the potential to enhance the efficacy and applicability of this formulation in the treatment of individuals afflicted with joint inflammatory illnesses.

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None

Conflict of Interest

None

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