Topical Anti-Inflammatory Formulations from Medicinal Plant Extracts: Stability, Efficacy, and Cytokine Modulation in a Carrageenan-Induced Paw Edema Model

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Abstract

Background: Traditional anti-inflammatory drugs, like NSAIDs, often cause side effects, which has fueled interest in plant-based alternatives with fewer adverse effects. Medicinal plants such as turmeric (Curcuma longa), ginger (Zingiber officinale), frankincense (Boswellia serrata), ashwagandha (Withania somnifera), and gotu kola (Centella asiatica) are known for their bioactive compounds with anti-inflammatory properties. This study aimed to evaluate the anti-inflammatory efficacy of these plant extracts in topical formulations. Methods: Ethanol extracts of the selected plants were prepared and formulated into topical gel and cream forms. Stability testing was conducted over a 30-day period to confirm the formulations' consistency, color, and pH. Anti-inflammatory efficacy was assessed using a carrageenan-induced paw edema model in Wistar rats. The animals were divided into six groups, including control, standard drug (indomethacin), and plant-based formulations at two extract concentrations (5% and 10%). Edema reduction was measured using a plethysmometer, and serum cytokine levels (TNF-α and IL-6) were quantified via ELISA. Histopathological analyses of tissue samples further evaluated the anti-inflammatory effects. Results: Both gel and cream formulations demonstrated stability over 30 days. The 10% extract cream formulation achieved the highest reduction in paw edema (70%) and reduced inflammation levels comparable to indomethacin. The 10% extract gel showed similar results (65% reduction). Biochemical analysis revealed significant reductions in TNF-α and IL-6 levels, especially in the 10% cream and gel groups, aligning with histopathological findings that indicated reduced tissue damage and inflammatory cell infiltration in treated groups compared to controls. Conclusion: The findings suggest that these plant extracts possess potent anti-inflammatory effects when formulated into stable, topical products. The 10% extract cream formulation was particularly effective, offering a promising alternative for managing inflammation with fewer side effects.

Keywords: Anti-inflammatory, plant extracts, topical formulation, cytokine modulation, carrageenan-induced edema

Significance | This study showed the potential of plant-based creams and gels as stable, effective, topical anti-inflammatory agents, reducing cytokine-induced inflammation.

1. Introduction

Inflammation is a vital response by vascular tissues to harmful stimuli, such as infections, irritants, and damaged cells. This defense mechanism involves immune cells, blood vessels, and chemical mediators that coordinate to protect the body.

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Editor Loiy Elsir Ahmed Hassan, Ph.D., And accepted by the Editorial Board October 15, 2024 (received for review August 04, 2024)

Please Cite This:
Raafat M. Alaatabi, Falah Hassan Shari, Mrs.Poulami Sen, Gunawan Widjaja (2024). "Topical Anti-Inflammatory Formulations from Medicinal Plant Extracts: Stability, Efficacy, and Cytokine Modulation in a Carrageenan-Induced Paw Edema Model", Journal of Angiotherapy, 8(10),1-6,9998

Inflammation can be categorized as acute or chronic; the former is crucial for healing wounds, while the latter is associated with various diseases, including cancer, cardiovascular disorders, and arthritis (Aggarwal & Harikumar, 2009). Unfortunately, chronic inflammation management often relies on traditional anti-inflammatory drugs, such as NSAIDs, which can have adverse side effects like cardiovascular risks and gastrointestinal complications (Balkwill, 2009). This has spurred interest in identifying natural anti-inflammatory agents that offer therapeutic benefits without significant side effects.

For centuries, medicinal plants have been a cornerstone of traditional medicine globally, valued for their bioactive compounds that confer anti-inflammatory effects. Flavonoids, alkaloids, terpenoids, and phenolic acids are among the compounds found in plants that contribute to their medicinal properties (Dhama et al., 2018). Research suggests that these bioactive compounds can reduce inflammation through multiple pathways, such as lowering oxidative stress, inhibiting pro-inflammatory enzymes, and reducing cytokine production (Siddiqui, 2011). For example, turmeric (Curcuma longa), ginger (Zingiber officinale), frankincense (Boswellia serrata), ashwagandha (Withania somnifera), and gotu kola (Centella asiatica) are traditionally known for their anti-inflammatory properties (Chrubasik, Pittler, & Roufogalis, 2005).

The purpose of this study is to explore the anti-inflammatory effects of extracts from these plants by developing topical formulations, such as gels and creams, and assessing their pharmacological activities. The formulations undergo evaluation for anti-inflammatory efficacy using a carrageenan-induced paw edema model in rats, which is a well-established method to simulate acute inflammation and assess early and late phases of inflammatory responses (Brinkhaus, Lindner, Schuppan, & Hahn, 2000). This model is complemented by biochemical assays and histopathological analysis to provide deeper insights into the mechanisms of inflammation inhibition.

The experimental process involves selecting five medicinal plants with known anti-inflammatory properties, extracting their active compounds, and formulating these extracts into stable topical products. The study includes an ethanol-based extraction, followed by the development of gel and cream formulations. The gel uses carbopol 940 as a gelling agent, while the cream is formulated as an oil-in-water emulsion. The anti-inflammatory potential of these formulations is tested by applying them to carrageenan-injected rat paws and measuring inflammation reduction using a plethysmometer at intervals (Siddiqui, 2011; Brinkhaus et al., 2000). Additionally, serum levels of cytokines TNF-α and IL-6, key markers of inflammation, are measured to validate the efficacy of these plant extracts further.

Histopathological analysis provides additional evidence by examining tissue samples from treated and control groups, revealing reduced tissue damage and inflammatory cell infiltration in treated samples (Mishra, Singh, & Dagenais, 2000). This research aims to offer safer, plant-based alternatives for managing chronic inflammatory conditions, leveraging traditional plant knowledge through scientific approaches. By identifying and characterizing plant-derived anti-inflammatory formulations, this study has the potential to advance natural therapeutic options with minimal side effects.

2. Materials and Methods

2.1 Plant Extract Selection and Preparation

Five medicinal herbs with established anti-inflammatory properties were selected for this study: turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), frankincense (*Boswellia serrata*), ashwagandha (*Withania somnifera*), and gotu kola (*Centella asiatica*). Fresh plant materials were collected and authenticated by a botanist. After verification, plant parts were air-dried at ambient temperature to preserve bioactive compounds. The dried materials were then ground into a fine powder. Ethanol was used as the solvent for extraction in a Soxhlet apparatus, which provided continuous extraction over an 8-hour cycle. Extracts were subsequently concentrated using a rotary evaporator at low pressure to remove excess solvent, and the concentrated extracts were stored at 4°C until required for further formulation.

2.2 Formulation Development

The ethanol extracts were incorporated into topical gel and cream formulations for testing.

2.2.1 Gel Formulation: A gel was created using carbopol 940 as the primary gelling agent. Carbopol 940 was dispersed in distilled water, neutralized with triethanolamine to achieve a stable gel consistency, and then the plant extract was incorporated. The mixture was stirred until homogenous.

2.2.2 Cream Formulation: A standard oil-in-water emulsion technique was used to develop the cream formulation. The oil phase consisted of stearic acid, cetyl alcohol, and mineral oil, while the aqueous phase included distilled water, glycerin, and the plant extract. Each phase was separately heated to 70°C, then combined with continuous stirring until a uniform emulsion formed as the mixture cooled.

Both formulations were assessed for physical stability (consistency, color, and pH) over a 30-day period and stored in sterile containers at ambient temperature.

2.3 Pharmacological Assessment

2.3.1Animal Ethics Approval

The anti-inflammatory efficacy of the formulations was tested using male and female Wistar rats (200-250 g). The rats were housed under controlled laboratory conditions with a 12-hour light/dark

cycle and ad libitum access to food and water. All procedures were approved by the Institutional Animal Ethics Committee and adhered to ethical guidelines for animal research.

2.3.2 Carrageenan-Induced Paw Edema Model

To evaluate the anti-inflammatory activity of the formulations, the carrageenan-induced paw edema model was utilized. Rats were divided into six groups (n=6 per group) as follows:

- Group I: Control (saline only)
- Group II: Standard anti-inflammatory drug (indomethacin 10 mg/kg)
- Group III: Gel formulation with 5% w/w plant extract
- Group IV: Gel formulation with 10% w/w plant extract
- Group V: Cream formulation with 5% w/w plant extract
- Group VI: Cream formulation with 10% w/w plant extract

A 0.1 mL injection of a 1% carrageenan solution was administered into the subplantar region of the right hind paw to induce acute inflammation. Topical application of the test formulations and reference drug was conducted one hour prior to the carrageenan injection. Paw edema volume was measured using a plethysmometer at baseline and at 1, 2, 3, and 4 hours post-carrageenan injection.

2.4 Biochemical Analysis

At the end of the experiment, blood samples were collected from the retro-orbital plexus to measure serum levels of pro-inflammatory cytokines (TNF-α and IL-6) using enzyme-linked immunosorbent assay (ELISA) kits. All procedures followed the manufacturer's instructions.

2.5 Histopathological Studies

Rats were euthanized at the conclusion of the study, and tissue samples from the inflamed paws were fixed in 10% formalin. Samples were processed using standard histological techniques, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Light microscopy was used to evaluate tissue architecture, inflammatory cell infiltration, and any additional indicators of inflammation.

2.6 Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare differences between groups. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Extract Yields and Formulation Stability

The ethanol extraction yield for each selected medicinal plant ranged from 5% to 15% of the initial dry weight. The gel and cream formulations demonstrated consistent physical stability over a 30-day evaluation period. Both formulations maintained their initial consistency, color, and pH without any observable phase separation or signs of microbial contamination.

3.2 Anti-inflammatory Activity

The anti-inflammatory potential of the gel and cream formulations was assessed using the carrageenan-induced paw edema model in rats. Paw edema reduction was observed across all treated groups, with a time-dependent decrease in swelling. As shown in Table 1, the 10% extract cream formulation demonstrated the highest reduction in paw edema, achieving levels comparable to the standard drug, indomethacin, at 4 hours post-injection. Specifically, the 10% extract cream and gel formulations achieved 70% and 65% edema reduction, respectively, at the 4-hour mark, indicating strong anti-inflammatory efficacy.

Table 1 shows a clear trend in edema reduction, with all formulations outperforming the control group at each time point. Notably, the gel and cream formulations with higher concentrations (10% extract) demonstrated superior performance compared to the lower concentrations (5% extract).

3.3 Biochemical Analysis

The levels of pro-inflammatory cytokines (TNF-α and IL-6) were significantly reduced in the treated groups relative to the control, as indicated in Table 2. In particular, the 10% extract cream and standard drug groups showed the greatest reductions in both TNF-α and IL-6 levels, achieving values close to those observed with indomethacin treatment. The gel formulation with 10% extract also significantly reduced cytokine levels compared to the control group, indicating its effective anti-inflammatory action ($p < 0.05$).

3.4 Histopathological Studies

Histopathological analysis revealed significant differences in tissue structure and inflammatory cell infiltration between the control and treatment groups. As summarized in Table 3, the control group exhibited severe inflammatory cell infiltration and extensive tissue damage, marked as ++++. In contrast, both the 10% extract gel and cream formulations showed only minimal infiltration and tissue damage (graded as +), comparable to the effects seen with indomethacin treatment. Lower-concentration formulations (5% extract) also displayed reduced inflammation but to a lesser extent.

The results from the carrageenan-induced paw edema model, biochemical assays, and histopathological evaluations collectively confirm the anti-inflammatory effectiveness of both gel and cream formulations. The 10% extract cream formulation, in particular, demonstrated the most potent anti-inflammatory effects across all metrics, making it a promising candidate for topical anti-inflammatory applications.

4. Discussion

This study investigated the anti-inflammatory potential of selected plant extracts formulated into topical gels and creams, with encouraging results supporting their efficacy. Both formulations

Table 1. Paw Edema Reduction (%) at Different Time Points

Group	1 hour	2 hours	3 hours	4 hours
Control (saline)	0%	0%	0%	0%
Standard drug (indomethacin)	35%	50%	60%	70%
Gel formulation (5% extract)	20%	35%	45%	55%
Gel formulation (10% extract)	30%	45%	55%	65%
Cream formulation (5% extract)	25%	40%	50%	60%
Cream formulation (10% extract)	35%	50%	60%	70%

Table 2. Serum Levels of Pro-inflammatory Cytokines

Group	TNF-α (pg/ml)	IL-6 (pg/ml)
Control (saline)	80 ± 5	90 ± 6
Standard drug (indomethacin)	30 ± 4*	35 ± 5*
Gel formulation (5% extract)	50 ± 5*	60 ± 5*
Gel formulation (10% extract)	40 ± 4*	50 ± 4*
Cream formulation (5% extract)	45 ± 5*	55 ± 5*
Cream formulation (10% extract)	30 ± 3*	35 ± 4*

*(*p < 0.05 compared to control group)*

Table 3. Histopathological Scores

Group	Inflammatory Cell Infiltration	Tissue Damage
Control (saline)	++++	++++
Standard drug (indomethacin)	+	+
Gel formulation (5% extract)	++	++
Gel formulation (10% extract)	+	+
Cream formulation (5% extract)	++	++
Cream formulation (10% extract)	+	+

(Note:** ++++ = **severe,** +++ = **moderate,** ++ = **mild,** + = **minimal)

demonstrated significant anti-inflammatory effects in the carrageenan-induced paw edema model, a widely accepted method for assessing anti-inflammatory activity. The reductions in paw edema across different formulations underscore the potential of these plant-based treatments as viable topical anti-inflammatory agents.

The anti-inflammatory properties observed align with previous research on the bioactive compounds in these plants. For instance, curcumin, the primary compound in Curcuma longa (turmeric), is known to inhibit inflammatory enzymes and cytokines, effectively modulating inflammatory responses, Zingiber officinale (ginger) contains gingerols and shogaols, compounds that inhibit the NF-κB signaling pathway, which plays a critical role in inflammation. The fim this study reinforce the potential of turmeric and ginger extracts in attenuating inflammation when applied topically.

Both the gel and cream formulations exhibited good stability, retaining their consistency, color, and pH over a 30-day period without microbial contamination. This stability enhances their practicality for clinical or over-the-counter use as topical applications. Notably, the 10% extract cream formulation achieved the highest anti-inflammatory effect, comparable to the standard drug, indomethacin, suggesting that increasing plant extract concentrations enhances efficacy. This aligns with previous findings on the dose-dependent effects of bioactive plant compounds.

Biochemical analyses further validated the anti-inflammatory action of the formulations. Significant reductions in TNF-α and IL-6, key pro-inflammatory cytokines, were observed in the treated groups compared to the control (Table 2). These cytokines are well-established mediators of inflammatory pathways and are frequently targeted in anti-inflammatory therapies. The 10% extract which showed the highest efficacy in reducing edema, also exhibited the most substantial decrease in TNF-α and IL-6 levels, supporting its superior anti-inflammatory potential.

Histopathological examination offered additional evidence of anti-inflammatory effects. The treated groups, particularly those receiving the 10% extract formulations, showed reduced inflammatory cell infiltration and minimal tissue damage, contrasting sharply with the control group (Table 3). This histological improvement is consistent with findings that plant-based therapies can effectively reduce tissue inflammation and preserve tissue integrity.

The bioactivity of thes is attributed to their diverse array of anti-inflammatory compounds. For instance, Boswellia serrata (frankincense) contains boswellic acids, known inhibitors of leukotriene synthesis, a key inflammatory mediator.

Additionally, *Withania somniferandha) and Centella asiatica (gotu kola) contain withanolides and asiaticosides, respectively, both known to exhibit anti-inflammatory effects. The combination of these bioactive constitutes to the significant anti-inflammatory properties observed in this study.

While the findings are promising, this study has limitations. The anti-inflammatory effects were only evaluated using a single animal model, necessitating further studies in different models and, eventually, in clinical settings to generalize the results. Additionally, the exact molecular mechanisms by which these extracts exert their anti-inflammatory effects remain to be fully elucidated. Future studies should focus on identifying and isolating the active compounds in these plants, as well as understanding their specific molecular targets and pathways.

The study supports the potential of these plant extracts as effective topical anti-inflammatory agents, especially in the form of creams and gels. With further research, these formulations could offer a natural, plant-based alternative for managing inflammatory conditions.

5. Conclusion

In conclusion, the selected plant extracts demonstrated significant anti-inflammatory activity when formulated into topical gels and creams. The 10% extract cream formulation was particularly effective, showing comparable results to the standard drug indomethacin. In contrast to traditional anti-inflammatory medications, plant-based formulations may have fewer adverse effects and might be a viable option for treating inflammatory disorders, according to these results.

Author contributions

R.M.A., F.H.S., and P.S. contributed to the conceptualization and design of the study. R.M.A. and F.H.S. carried out the data collection and analysis. G.W. contributed to data interpretation and provided critical revisions to the manuscript. All authors reviewed and approved the final version of the manuscript for publication.

Acknowledgment

The authors were grateful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Aggarwal, B. B., & Harikumar, K. B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. The International Journal of Biochemistry & Cell Biology, 41(1), 40-59.

Balkwill, F. (2009). Tumour necrosis factor and cancer. Nature Reviews Cancer, 9(5), 361-371.

Brinkhaus, B., Lindner, M., Schuppan, D., & Hahn, E. G. (2000). Chemical, pharmacological, and clinical profile of the East Asian medical plant Centella asiatica. Phytomedicine, 7(5), 427-448.

Cheng, A. L., Hsu, C. H., Lin, J. K., et al. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Research, 21(4B), 2895-2900.

Chrubasik, S., Pittler, M. H., & Roufogalis, B. D. (2005). Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles. Phytomedicine, 12(9), 684-701.

Cragg, G. M., & Newman, D. J. (2005). Plants as a source of anti-cancer agents. Journal of Ethnopharmacology, 100(1-2), 72-79.

Dhama, K., Karthik, K., Khandia, R., et al. (2018). Medicinal and therapeutic potential of herbs and plant metabolites/extracts countering viral pathogens-current knowledge and future prospects. Current Drug Metabolism, 19(3), 236-263.

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008). Curcumin as "Curecumin": From kitchen to clinic. Biochemical Pharmacology, 75(4), 787-809.

Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2012). Therapeutic roles of curcumin: Lessons learned from clinical trials. The AAPS Journal, 15(1), 195-218.

Hatcher, H., Planalp, R., Cho, J., Torti, F. M., & Torti, S. V. (2008). Curcumin: From ancient medicine to current clinical trials. Cellular and Molecular Life Sciences, 65(11), 1631-1652.

Jiang, H., Somogyi, A., Jacobsen, N. E., Timmermann, B. N., & Gang, D. R. (2006). Analysis of curcuminoids by positive and negative electrospray ionization and tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 20(7), 1001-1012.

Khor, T. O., Keum, Y. S., Lin, W., et al. (2006). Combined inhibitory effects of curcumin and phenethyl isothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice. Cancer Research, 66(2), 613-621.

Kunnumakkara, A. B., Anand, P., & Aggarwal, B. B. (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. Cancer Letters, 269(2), 199-225.

Lantz, R. C., Chen, G. J., Sarihan, M., et al. (2005). The effect of turmeric extracts on inflammatory mediator production. Phytomedicine, 12(6-7), 445-452.

Maheshwari, R. K., Singh, A. K., Gaddipati, J., & Srimal, R. C. (2006). Multiple biological activities of curcumin: A short review. Life Sciences, 78(18), 2081-2087.

Mishra, L. C., Singh, B. B., & Dagenais, S. (2000). Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): A review. Alternative Medicine Review, 5(4), 334-344.

Siddiqui, M. Z. (2011). Boswellia serrata, a potential anti-inflammatory agent: an overview. Indian Journal of Pharmaceutical Sciences, 73(3), 255.