In-Vivo Testing of Anti-Inflammatory Activity of Panyawan Topical Spray

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Abstract

Background: Inflammation is a common medical concern, and alternative, cost-effective treatments are being explored. Conventional anti-inflammatory drugs, such as NSAIDs, can be expensive and may cause adverse effects. Panyawan (*Tinospora rumphii*) is a medicinal plant recognized for its anti-inflammatory properties, making it a promising alternative treatment option.

Objective: This study evaluates Panyawan topical spray's efficacy in reducing acute inflammation in a rat paw edema model

Methods: The study evaluated varying concentrations (10%, 5%, and 1% w/v) of Panyawan topical spray against Diclofenac sodium spray and a placebo. A total of 15 male albino rats were randomly assigned to five groups. Paw edema was induced using carrageenan, and paw size was measured at baseline and intervals up to 12 hours post-treatment.

Results: Results indicated that all treatment groups reached baseline paw size, with the 5% Panyawan spray exhibiting the fastest response by the 8th hour, followed by the 1% and 10% Panyawan and Diclofenac sodium sprays at the 10th hour, and the placebo at the 12th hour.

Conclusion: The findings suggest that 5% Panyawan spray has the fastest anti-inflammatory response across three concentrations. However, all treatments are comparable to diclofenac at 10 and 12 hours. Further research is recommended to optimize formulation and enhance therapeutic application.

Keywords: *Tinospora rumphii, Panyawan Stem Extract, Panyawan Topical Spray, Male Albino Rat, Inflammation, Spray Concentration*

Acknowledgments: The authors would like to express their sincere gratitude to their advisers, the panel of reactors, the questionnaire validators, and the Dean of the College of Pharmacy and Chemistry.

Funding/support: None

Conflicts of interest: None

Introduction

Inflammation is the body's natural response to harmful stimuli, but it can lead to complications if untreated. If left unmanaged, chronic inflammation contributes to various health conditions, including arthritis and cardiovascular diseases (Ferrero-Miliani et al., 2007). Conventional anti-inflammatory drugs, such as NSAIDs, are effective but costly and sometimes associated with side effects, including gastrointestinal issues and cardiovascular risks (Rehman et al., 2023).

In the Philippines, high drug prices prevent many individuals from obtaining necessary medications (Department of Health, 2019). Yee (2019) reported that 99% of Filipinos refrain from purchasing all their prescribed medications due to high costs, which can negatively impact health outcomes. To address these concerns, herbal alternatives such as Panyawan (*Tinospora rumphii*) are being explored.

Panyawan, also known as Makabuhay, has been used in traditional medicine for its therapeutic properties for a long time. Studies indicate that Panyawan contains flavonoids, alkaloids, glycosides, and steroids, which exhibit anti-inflammatory, antioxidant, and immunomodulatory properties (Ahmad et al., 2016; Upadhya et al.,

2011). These compounds inhibit inflammatory mediators such as prostaglandins and cytokines, reducing swelling and pain (Raghu et al., 2009). Research on related species, such as Tinospora cordifolia, has shown promising results in managing acute and chronic inflammation (Ibaviosa et al., 2019).

Given the limited research on Panyawan's efficacy in topical formulations, this study aims to determine the effectiveness of Panyawan topical spray in reducing inflammation. The findings may offer a more accessible and cost-effective alternative to commercial anti-inflammatory treatments.

Materials and Methods

Research Design

An experimental research design was utilized to determine the anti-inflammatory effects of Panyawan topical spray.

Research Locale

The study was conducted at the San Pedro College Animal Lab and Research Hub in Davao City, Philippines.

Plant Material Collection and Extraction

Panyawan stems were collected from Malagos, Baguio District, Davao City, and authenticated by a forester from the Department of Environment and Natural Resources, Community Development and Natural Resources Office. The extraction process involved maceration of 400 g panyawan stems in 500 ml 95% ethanol for 48 hours, followed by rotary evaporation.

Albino Rat Source

Male albino rats weighing between 175 and 260 g were obtained from a certified breeder. They were housed in a controlled environment with food and water ad libitum.

Topical Spray Formulation

The Panyawan topical spray was prepared in three concentrations (1%, 5%, 10% w/v) using ethanol as a vehicle. Other excipients included propylene glycol, lecithin, citric acid, polyvinyl pyrrolidone, and peppermint oil.

In Vivo Anti-inflammatory Bioassay

The experiment randomly assigned fifteen male albino rats into five treatment groups: 10% Panyawan, 5% Panyawan, 1% Panyawan, Diclofenac sodium spray (positive control), and placebo spray. A 0.1 ml dose of 1% carrageenan solution induced inflammation in the right hind paw of each rat. After five hours, when inflammation peaked, the assigned topical spray was applied every four hours. Paw size measurements were taken with a Vernier caliper at baseline and at regular intervals up to 12 hours after treatment.

Ethical Considerations

The University of the Immaculate Conception approved the study, as per the Institutional Animal Care and Use Committee (IACUC), with Protocol Code 00224, dated April 14, 2024. It adhered to the Republic Act No. 8485 (Animal Welfare Act of 1998).

Results

All treatment groups showed a reduction in inflammation over time (Table 1). The 5% Panyawan spray achieved complete edema reduction by the 8th hour, while 1% and 10% Panyawan and Diclofenac sodium spray reached baseline by the 10th hour. The placebo group required 12 hours.

Table 1.
Percent Reduction of Carrageenan-induced Edematous Albino Rat Paws in 12 hours

Treatment	Percent reduction in paw edema (Mean±SD)						
	0 hr	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs
10% Panyawan	0±0.00	51±0.22	70±0.26	70±0.26	78±0.2	100±0.00	100±0.00
5% Panyawan	0±0.00	51±0.22	81±0.17	81±0.17	100±0.00	100±0.00	100±0.00
1% Panyawan	0±0.00	41±0.08	64±0.17	64±0.17	88±0.11	100±0.00	100±0.00
Diclofenac sodium spray	0±0.00	53±0.06	83±0.29	83±0.29	88±0.11	100±0.00	100±0.00
Placebo spray	0±0.00	51±0.22	66±0.15	77±0.25	92±0.14	92±0.14	100±0.00

All treatment groups exhibited a reduction in paw edema over the 12-hour observation period. The 5% Panyawan spray demonstrated the fastest anti-inflammatory response, achieving baseline paw size by the 8th hour. This was followed by the 1% and 10% Panyawan sprays and Diclofenac sodium spray, which reached complete reduction by the 10th hour. The placebo group required the entire 12-hour period to return to baseline.

Among the Panyawan formulations, the 5% spray consistently showed higher percent reduction at earlier intervals, indicating a more efficient and sustained response. Despite being more concentrated, the 10% spray did not outperform the 5%, suggesting that increasing the concentration beyond an optimal level does not necessarily enhance effectiveness. The 1% formulation showed slower progression, reflecting a dose-dependent trend.

The Diclofenac sodium spray, used as the positive control, performed comparably to the 5% Panyawan spray by the 10th hour. In contrast, the placebo group exhibited the slowest resolution, confirming that the observed anti-inflammatory effects were due to the active compounds. Overall, the results suggest that the 5% Panyawan formulation is the most effective concentration for reducing inflammation within the tested timeframe.

Discussion

The 5% Panyawan spray exhibited the most effective and rapid anti-inflammatory action, achieving complete edema reduction by the 8th hour, faster than both the 10% and 1% formulations. Its performance was comparable to the Diclofenac sodium spray, which achieved the same effect by the 10th hour, indicating that the 5% concentration provided optimal efficacy. In contrast, the placebo spray showed the slowest response, with complete recovery observed only at 12 hours. These results suggest that Panyawan possesses significant anti-inflammatory properties, with the 5% formulation demonstrating the most efficient dose-response effect.

The lower efficacy of the 10% Panyawan spray compared to the 5% formulation may be due to a saturation effect, where excessive amounts of the active compound reduce skin absorption or bioavailability (Sousa et al., 2021; Fang et al., 2001). Similar patterns are observed in other plant-based formulations, where an optimal concentration yields the most effective result. At higher levels, the active ingredients may also interact with other components of the formulation, affecting how well the compound is absorbed and distributed.

Panyawan's anti-inflammatory action is attributed to its rich phytochemical profile, which includes flavonoids, alkaloids, glycosides, and steroids. These bioactive compounds have been reported to modulate inflammatory mediators such as cytokines and prostaglandins, thereby reducing swelling and pain (Upadhya et al., 2011; Raghu et al., 2009). The presence of flavonoids, such as quercetin and kaempferol, in Tinospora species has been linked to antioxidant and anti-inflammatory properties, as they inhibit the release of pro-inflammatory cytokines and reduce oxidative stress (Ibaviosa et al., 2019).

In addition to its pharmacological activity, the topical spray formulation plays a crucial role in delivering the drug. Ethanol, used as a vehicle in the Panyawan topical spray, enhances skin penetration, thereby improving bioavailability (Pawar & Chaudhari, 2015). The inclusion of propylene glycol and lecithin as excipients further supports drug absorption by increasing skin permeability and ensuring the uniform distribution of the active ingredients.

Another possible explanation for the variations in effectiveness among different concentrations is the impact of vehicle components on the drug's diffusion. Studies on topical NSAIDs suggest that permeation

enhancers and stabilizers influence the rate of drug release and absorption (Lin et al., 2024). This could explain why the 5% formulation achieved faster and more consistent results than the 10% formulation, which may have been hindered by oversaturation.

Compared with previous research, the present study's findings are consistent with those of Gatmaitan et al. (2023), who reported significant anti-inflammatory effects of *Tinospora cordifolia* in animal models, with paw edema reduction occurring within 6 to 12 hours after administration. However, while Gatmaitan et al. focused on a different species and systemic treatment, the current study evaluated *Tinospora rumphii* in a topical spray formulation, which achieved complete edema reduction by the 8th hour—indicating a faster and formulation-dependent response. Similarly, the results align with those of Ibaviosa et al. (2019), who demonstrated that *T. rumphii* possesses both antimicrobial and anti-inflammatory properties comparable to those of standard pharmacological treatments. Unlike Ibaviosa et al. (2019), who examined general extract activity, the present study highlights explicitly the optimized 5% Panyawan spray as an adequate topical anti-inflammatory preparation, reinforcing *T. rumphii's* therapeutic potential and its viability as a natural alternative to synthetic agents.

Overall, the results suggest that the 5% Panyawan spray formulation provides an effective, natural alternative for inflammation management with comparable efficacy to commercially available Diclofenac sodium spray. Future research should focus on optimizing formulation stability, exploring potential synergistic effects with other natural anti-inflammatory agents, and conducting human clinical trials to validate the therapeutic potential of Panyawan in dermatologic and musculoskeletal inflammatory conditions.

Limitations

This study was limited by its small sample size and the use of an animal model that may not fully reflect the complexities of human inflammation. The evaluation was restricted to a single-dose application in an acute inflammation setting, without assessing long-term safety, formulation stability, or varied dosing regimens. Additionally, only Diclofenac sodium was used as a comparator, and the study did not explore the underlying molecular mechanisms of Panyawan's anti-inflammatory effects.

Conclusion

The study confirms that Panyawan topical spray has significant anti-inflammatory effects, with 5% concentration demonstrating the highest efficacy. Its performance is comparable to Diclofenac sodium, suggesting its potential as a cost-effective alternative. Future research should focus on optimizing formulations and evaluating the long-term safety and pharmacokinetics of these compounds.

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