Original Research Article

In Vivo Evaluation of the Anti-Inflammatory Effects of *Tinospora rumphii* Topical Spray in a Carrageenan-Induced Rat Paw Edema Model

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ABSTRACT

This study investigated the anti-inflammatory effects of a topical spray containing *Tinospora rumphii* (Heavenly elixir) extract on carrageenan-induced paw edema in male rats. Inflammation is a persistent global health concern. Traditional medicine in the Philippines frequently uses herbal plants to address common illnesses, and *Tinospora rumphii* has been explored for its anti-inflammatory potential. The topical spray was developed with varying concentrations (1%, 5% and 10%) of *Tinospora rumphii* extract, along with other pharmaceutical components and ethanol as a vehicle. All treatment groups, the *Tinospora rumphii* formulations, a positive control (diclofenac sodium spray), and a placebo spray, successfully reduced paw edema, restoring the paw size to baseline during the observation periods of 2, 4, 6, 8, 10 and 12 hours. Results indicate significant differences within treatments over time, as well as notable differences among the treatments across the same period. While all achieved a full reduction in inflammation, the time required for this varied among the treatments. The *Tinospora rumphii* spray at a particular concentration demonstrated the quickest anti-inflammatory effect, reaching maximum reduction notably faster than other concentrations, the positive control, and the placebo. Specifically, one *Tinospora rumphii* concentration exhibited an anti-inflammatory effect comparable to the positive control in terms of the time taken for the paw edema to return to its original size. These findings indicate the efficacy of *Tinospora rumphii* as a viable anti-inflammatory medication. The results of the study will be a basis for future researchers to explore more about the properties of the plant as an anti-inflammatory.

Keywords: anti-inflammatory, topical spray, carrageenan-induced paw-edema

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1.0 Introduction

The body's immune system naturally responds to harmful stimuli like irritants and pathogens by causing inflammation, in which the effect will results to more complications if left untreated. Health organizations are continuously working to develop new, consumer-friendly solutions for inflammation.

High healthcare costs are a significant barrier to medication adherence, with a literature review of 79 articles, and increased cost-sharing was associated with worse adherence, persistence, or even discontinuation of the prescribed medications (1). In a study result which was conducted in the Philippines, Filipinos still continue to experience problems on the access of medicines despite the implementation of the government on Value-added Tax (VAT) exemption and reduced prices. This implies that there is a need to review existing policies and regulations of the government in order to improve the availability and accessibility of these medicines (2).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for their effective pain-relieving and anti-inflammatory properties; however, their use is often linked to gastrointestinal side effects, particularly among high-risk individuals (3). Herbal plants like capsaicin, arnica, bromelain, and curcumin are being investigated as alternatives. The natural, holistic remedies and use of herbal alternatives are the rising trend nowadays. This preference is evident in the increasing market for herbal supplements, often perceived as safer and more environmentally friendly than conventional pharmaceuticals, and the global herbal medicine market is forecasted to continue its growth.

The plant, Panyawan or Makubahay, is a common name of *Tinospora rumphii,* which is an indigenous plant found commonly in the forest. Its folkloric use is an anti-inflammatory, and it alleviates pain and swelling. Recognized as a Philippine herbal alternative medicine, Makabuhay (Panyawan) holds a significant place in local health practices (4). In vivo results of *Tinospora cordifolia* validated the in vitro results, and there was a significant reduction in serum level of pro-inflammatory cytokines and mediators (5). Numerous pharmacology studies have demonstrated that other *Tinospora* specie, *T. cordifolia* modulates key signaling pathways related to cell proliferation, inflammation, and immunomodulation (6). Studies also suggest that various doses of *Tinospora rumphii* extract effectively reduce paw edema in rat paw edema assays by inhibiting inflammatory mediators and prostaglandins (7).

Tinospora rumphii belongs to the family Menispermaceae, is a large, glabrous, deciduous climbing shrub that is distributed in Southeast Asia (8). This plant is well-known for its therapeutic qualities and is utilized to cure a range of illnesses, with numerous potential health and mental advantages. They have inherent analgesic qualities that help reduce pain from headaches, muscular aches, and arthritis. Its stem is a main component of remedies.

The plant, *Tinospora rumphii*, contains phytochemicals such as triterpenes, diterpenes, lactones, alkaloids, flavonoids, and flavone glycosides. These compounds have pharmacological functions as antioxidants, anti-diabetics, immunomodulators, and anti-inflammatories (9). The novel aspect of this research lies in its investigation into the efficacy of *Tinospora rumphii* when delivered via a topical spray formulation. Spray formulations allow for direct application to the affected area and provide localized action. While the plant is known for its therapeutic qualities and anti-inflammatory properties,

the limited study of its herbal anti-inflammatory activity, particularly in a topical spray, necessitates further research to ensure its effectiveness in addressing inflammation issues. This particular study specifically aims to determine the anti-inflammatory activity of *Tinospora rumphii* topical spray on carrageenan-induced paw edema in Wistar rats.

2.0 Materials and Methods

2.1 Plant material collection and extraction

In Purok 5 Malagos, Baguio district, Davao City, Philippines, the stem of *Tinospora rumphii* was harvested (Fig. 1). This was stored in a dry, hygienic container. A certificate of authenticity from Davao City's Department of Environmental and Natural Resources was obtained. The stem was macerated in 95% ethanol for 48 hours, and the solvent was evaporated using a rotary evaporator.

2.2 Topical spray formulation

Three varying dosage strengths (in w/v) of *Tinospora rumphii* topical sprays were formulated (1%, 5%, and 10%). This is based on the study of Fernandez *et al*. (2021) (10), Ethanol, peppermint oil, citric acid, polyvinyl pyrrolidone, lecithin, propylene glycol, and isopropyl myristate are the ingredients in the spray formulation.

2.3 In vivo anti-inflammatory bioassay

In the bioassay, 15 male Wistar rats weighing 175-260 grams were acclimatized and housed in an animal hub. The study was reviewed by the UIC Research and Ethics Committee, having the Protocol Code of UG-0036-02-24. The researchers complied with the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) for the provision of housing, food, water, ventilation,

Figure 1: Stem of *Tinospora rumphii*.

and other routine care procedures for animals. All of these factors are vital in protecting the rights of the subjects, enhancing the validity of research outcomes, and upholding scientific or academic integrity. The rats were placed inside an individually ventilated cage (IVC) with their own feeding bottles. With regard to the food and water, animals were fed a commercially available rodent diet.

Prior to the administration of the treatment to the test animals, the size of their right hind paw was measured in millimeters using a vernier caliper, one hour before the treatment. To induce inflammation, about 0.1 ml of a 1% carrageenan solution is administered into the subplantar region of the right hind paw of each test animal (Fig. 2). The observations of inflammation were made during the first hour after the injection, which serves as the reference for subsequent hours of observation. Starting from this time point up to the fifth hour, the paw size was measured. The maximum sizes of paws found in the fifth hour are used as the baseline for the subsequent procedure. Immediately after successfully inducing edema on the paw of Wistar rats and after measuring its size, the topical sprays were applied to the site of the inflamed paw. The three formulated *Tinospora rumphii* topical sprays with varying dosage strengths were applied to the test control group containing three rats, respectively. The diclofenac sodium spray, which is commercially available and is frequently used as a first-line treatment for inflammation and pain was applied, grouped as the positive control (n=3) and the placebo spray, which contains the excipients of the formulated spray without the extract, was applied to the placebo control group (n=3). Using three test animals per treatment group in this anti-inflammatory study is scientifically and ethically justified. This aligns with OECD guidelines and the 3Rs principle of reduction. The study's design involves multiple time points (2 to 12 hours), which requires repeated handling.

All topical sprays were administered every four hours for a day. Further, the size of edema in the paw of each rat was measured using a vernier caliper every two hours. The percentage reduction of the following sprays at a point in time was calculated using the formula below.

% Paw Reduction = (Initial Paw Size - Current Paw Size)/Range x 100%

Range = Largest Paw Size - Smallest Paw Size

2.4 Statistical Analysis

The data that were collected and were analyzed using the statistical tool, repeated measures analysis of variance (ANOVA).

3.0 Results

Table 1 shows the test of difference in the percentage reduction of the carrageenan-induced paw sizes within treatments over time.

It was observed that the paw size was found to decrease more quickly with the 5% *Tinospora rumphii* spray than with the 10% and 1% concentrations of the same spray. All of the data on the observed differences were found to be statistically significant, according to the statistical test used to compare the percentage reduction of paw size in carrageenan-induced rats at various time points within each treatment group.

The F-value for the group that received a 10% concentration of the plant extract was 7345.226. This high F-value suggests that the treatment, not chance, is responsible for the significant variation in the paw size reduction over time. The result is statistically significant, and the likelihood that it would occur by chance is less than one in a

thousand, according to the corresponding p-value of less than 0.001.

For the 5% concentration, the F-value for the group was 36494.909. The said value is even greater than that of the 10% group, indicating that the 5% spray had a longer-lasting and more substantial impact on paw size reduction. Additionally, the p-value was less than 0.001, confirming the result's high level of statistical significance.

While the group receiving the 1%, the F-value was 26306.435. Similar to the earlier findings, this suggests that the treatment has a significant long-term impact. The fact that the p-value stayed below 0.001 indicates that the differences are not the result of chance.

The F-value in the positive control group, which was given a typical anti-inflammatory treatment, was 21283.000. With a p-value of less than 0.001, this once more shows a highly significant difference in the reduction of paw size over time. On the other hand, the F-value in the placebo group, which was not given any active treatment, was 9039.122. With a p-value below 0.001, this indicates a statistically significant change in paw size over time, even though it is less than the other treatment groups. Instead of reflecting actual treatment efficacy, this could be a reflection of placebo effects or natural recovery.

Furthermore, it is noteworthy that all groups had exceptionally high effect sizes. The omega squared value of 1.000 supports this, showing that the treatment itself is responsible for almost all of the variation in paw size loss over time. A perfect effect size, which is exceedingly uncommon and indicates a very potent treatment effect, is represented by an omega squared value of 1.000.

4.0 Discussion

The researcher formulated *Tinospora rumphii* sprays because spray formulations avoid first-pass metabolism, they have a quicker onset of action, higher bioavailability, and improved patient convenience, particularly for people who have trouble swallowing.

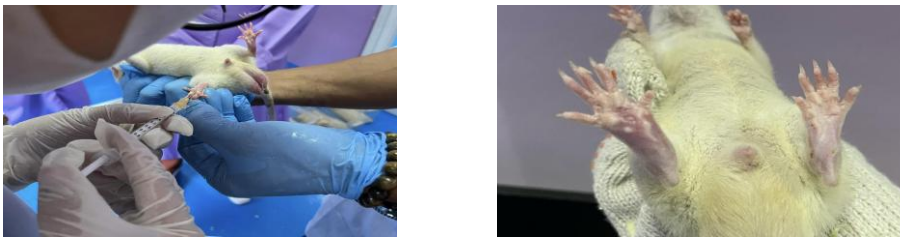
The results showed that all the groups had a similar decrease in inflammation in the 2nd hour. However, the reduction continued to increase for the *Tinospora rumphii* treatment group (1% w/v, 5% w/v, 10% w/v) at a significant, gradual reduction compared to the placebo. As observed, only the placebo treatment reached a 100% reduction on the 12th hour, which signifies that it is the slowest treatment to reduce the inflammation in the paw of the rats.

The gradual decrease in the placebo group suggests that inflammation normally resolves, although more slowly in the absence of therapy, indicating the body's natural ability to control the inflammatory response and the factors contributing to it. Factors that may influence to the gradual reduction of paw size in the placebo group may be the excipients used in the formulation of the topical spray and the natural ability of the body to control acute inflammation as well as the limited time duration of the 1% carrageenan solution to produce acute inflammation, the injection of seaweed extract, carrageenan, subcutaneously into the hind paw in rats or mice causes local inflammation within about 4 hours and the inflammation normally lasts for up to 12 hours. Moreover, according to the study, given that carrageenan is diluted by distilled water, the water has the capacity to induce diuresis, which is a factor that results in inflammation to gradually reduces over time due to frequent urination of the rat as a response to the solution that had been used for the induction of inflammation. Therefore, it is expected that the inflammation normally resolves itself over time within twelve hours. Related studies showed that *Tinospora* has a significant reduction in serum level of pro-inflammatory cytokines and mediators in both in vivo and in vitro (5).

Table 1: Test of difference in the percentage reduction of the carrageenan-induced paw sizes of Wistar rats within treatments over time.

Treatment Type	% Paw reduction ($\bar{x} \pm SD$)								
	0h (baseline)	**2h**	**4h**	**6h**	**8h**	**10h**	**12h**	**F**	**ω^2**
10% *Tinospora rumphii* spray	0.00	51±0.22	70±0.26	70±0.26	78±0.20	100±0.00	100±0.00	7345.226*	1.000
5% *Tinospora rumphii* spray	0.00	51±0.22	81±0.17	81±0.17	100±0.00	100±0.00	100±0.00	36494.909*	1.000
1% *Tinospora rumphii* spray	0.00	41±0.08	64±0.17	64±0.17	88±0.11	100±0.00	100±0.00	26306.435*	1.000
Diclofenac sodium spray	0.00	53±0.06	83±0.29	83±0.29	88±0.11	100±0.00	100±0.00	21283.000	1.000
Placebo Spray	0.00	51±0.22	66±0.15	77±0.25	92±0.14	92±0.14	100±0.00	9039.122	1.000

** Significant at P< 0.001, post-treatment at 12 hours*



(a) Induction of inflammation. **(b) Inflamed rat's paw.**

Figure 2: Induction of carrageenan-induced inflammation in rats.

Several pharmacology studies have demonstrated that *Tinospora* species would act by modulating the key signaling pathways that are related to cell proliferation, inflammation, and immunomodulation (6). Studies also suggest that various doses of *Tinospora rumphii* extract effectively reduce paw edema in rat paw edema assays by inhibiting inflammatory mediators and prostaglandins (7).

Ten *Tinospor*a species have been investigated for their pharmacological activities, demonstrating notable effects such as neuroprotective and anti-neuroinflammatory activities, anti-inflammatory effects, anti-diabetic and anti-obesity properties, immunomodulatory functions, anticancer potential, larvicidal and antimalarial properties, and hepatoprotective effects (7).

Traditionally the plant was used for managing various ailments, its phytochemical analysis of *T. rumphii* reveals bioactive compounds such as triterpenes, flavonoids, alkaloids, and lactones, which contribute to its antioxidant, anti-diabetic, immunomodulatory, and anti-inflammatory properties (9).

In general, the results of the study indicate that the *Tinospora rumphii* plant extract has a property to be an alternative treatment to conventional anti-inflammatory medications. However, further research is recommended by the researcher to better understand its safety profile and the biological mechanisms underlying its anti-inflammatory effects.

5.0 Conclusion

All treatments have reached the rat's baseline paw size with a percent reduction of 100% in a 12-hour period of observation. However, the time to achieve a 100% reduction varied among the groups. These findings suggest that the 5% *Tinospora rumphii* spray may offer a favorable concentration for rapid and potential anti-inflammatory response, as it reached the fastest 100% reduction among the treatments. Results from the repeated measures ANOVA showed significant F-values for all treatment groups, which indicates that paw size consistently decreased over time within each treatment group. Omega squared values for all treatments signify a strong consistency in the time-related efficacy of the anti-inflammatory response of the treatment groups, regardless of concentration. The findings indicate that while all treatments have the potential to have anti-inflammatory properties, their rates of action differ significantly, with the 5% spray showing remarkable activity as the most rapid and anti-inflammatory formulation during the early phase of treatment. This study will serve as baseline data, and further research, such as histological analysis or cytokine profiling, is recommended to fully explore the extract's potential as an anti-inflammatory agent.

Authorship contribution statement

KBO and JVR: Draft correction, data analysis, review and editing. **JB, FFF and CML**: Methodology, data analysis.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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