Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.5647

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF FICUS BENJAMINA L USING CARRAGEENAN-INDUCED PAW EDEMA MODEL IN ALBINO RATS

Farzana Khan[1], Samina Afzal[2 *], Muhammad Hasnat Akhtar[3], Mahpara Laiq[4], Sunder Zahra[5], Huma Majeed[6], Benish Saeed[7], Muhammad Sheraz Qureshi[8], Hafiz Muhammad Usman Abid[9*]

[1,2 ,5 ,7]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University Multan, Punjab, Pakistan.
[1, 6]Bakhtawar Amin College of Pharmaceutical Sciences, Multan, Punjab, Pakistan
[3]CMH Kharian Medical College, Kharian, Pakistan
[4]Department of Pharmacy Practice, The Islamia University of Bahawalpur, Bahawalpur
[4, 5]Bahawalpur College of Pharmacy BMDC Complex, Punjab, Pakistan
[8.]Department of Pharmacognosy, Multan University of Science and Technology, Multan, Punjab, Pakistan.
[9*]Department of Pharmaceutics, Bahauddin Zakariya University, Multan, Punjab, Pakistan.

***Corresponding Authors**: Samina Afzal, Hafiz Muhammad Usman Abid
[*]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University Multan, Punjab, Pakistan. email: samina.afzal@bzu.edu.pk
[*]Department of Pharmaceutics, Bahauddin Zakariya University, Multan, Punjab, Pakistan
Email: usmanabid394@gmail.com

Abstract:
Inflammation plays a crucial role in immune responses and tissue repair processes, making the search for effective anti-inflammatory agent's paramount. Medicinal plants have long been recognized for their therapeutic potential in managing inflammatory conditions, necessitating further exploration of their pharmacological properties. Despite advancements in pharmaceutical research, there remains a need to identify novel anti-inflammatory agents with fewer side effects. Ficus benjamina L, a commonly found plant, has been traditionally used for various medicinal purposes, yet its anti-inflammatory properties warrant systematic investigation. This research aims to evaluate the anti-inflammatory activity of Ficus benjamina L through in-vivo methods, specifically utilizing the Carrageenan-induced paw edema model in albino rats. The study seeks to bridge the gap in knowledge regarding the therapeutic potential of Ficus benjamina L as a natural anti-inflammatory agent. The study involved the collection and preparation of methanolic extracts from Ficus benjamina L, followed by the administration of varying doses to albino rats. Anti-inflammatory activity was evaluated implying the Carrageenan-induced paw edema model, with diclofenac sodium as the standard reference. The methanolic extract of Ficus benjamina L demonstrated significant anti-inflammatory properties in a dose-dependent manner, resulting in a reduction of paw edema volume compared to the standard diclofenac sodium. Statistical analysis confirmed the reliability of the

findings. The study concludes that Ficus benjamina L exhibits potent anti-inflammatory activity, suggesting its therapeutic potential in managing inflammatory conditions.

Keywords: Anti-inflammatory activity, Ficus benjamina L, Carrageenan-induced paw edema model, Albino rats, Methanolic extract.

Introduction:

Inflammation is a pivotal aspect of the body's immune response, pivotal for shielding against tissue damage and infections. Nonetheless, when inflammation goes awry, it can pave the way for chronic ailments, underscoring the necessity for effective anti-inflammatory interventions[1]. Over time, medicinal plants have surfaced as promising reservoirs of natural compounds with therapeutic efficacy in managing inflammatory maladies[2]. Among these botanical treasures, Ficus benjamina L, commonly referred to as the weeping fig, has emerged for its traditional medicinal applications transcending various cultures. The historical heritage of medicinal plants in healing practices stretches back through centuries, with indigenous wisdom often safeguarding invaluable insights into their remedial virtues. Despite the ascent of modern pharmaceuticals, the relevance of traditional medicine endures, particularly in locales where access to conventional healthcare remains limited[3]. This reliance accentuates the imperative of scientifically scrutinizing the pharmacological attributes of medicinal plants to unlock their complete therapeutic potential.

Ficus benjamina L, a member of the Moraceae family, boasts a storied past of medicinal utility, notably in mitigating inflammatory afflictions[4]. Its diverse components, encompassing leaves, bark, and fruits, have been integral to traditional remedies targeting a spectrum of ailments from digestive maladies to dermatological issues. Yet, while anecdotal accounts hint at its anti-inflammatory prowess, a rigorous scientific endorsement is lacking, thus prompting a call for meticulous investigation. This study endeavors to bridge this gap by assessing the anti-inflammatory efficacy of Ficus benjamina L through in-vivo experimentation. Specifically, we employ the Carrageenan-induced paw edema model in albino rats, a widely recognized method for gauging anti-inflammatory properties[5]. Through systematic experimentation and statistical scrutiny, our goal is to delineate the magnitude of Ficus benjamina L's anti-inflammatory potential and juxtapose it with the benchmark pharmaceutical, diclofenac sodium. In this paper, we present a comprehensive scrutiny of our experimental outcomes, commencing with the materials and methods employed in plant extraction and animal experimentation. Subsequently, we delve into the findings, elucidating the discernible reduction in paw edema volume subsequent to treatment with Ficus benjamina L extract. Finally, we engage in a thorough discourse on the ramifications of our findings, accentuating the therapeutic promise of Ficus benjamina L as a natural anti-inflammatory agent while proposing avenues for future exploration.

Material and methods:

Plant Collection and Extract Preparation:

The Ficus benjamina L botanical specimen was obtained from the Allah Ditta Nursery Farm situated on the Northern Bypass, Boson Road in Multan. Authentication was conducted by Professor Dr. Zafar Ullah Zafar, an Associate Professor of Botany at Bahauddin Zakariya University, Multan, Pakistan. Upon procurement, the plant underwent meticulous washing with water to remove any surface impurities before being laid out on clean paper sheets for shade drying at room temperature (25°C). Subsequent to the shade drying process, the various plant components, including stems, roots, and leaves, were finely pulverized using an electric grinding mill, resulting in a coarse powder. This powder was then meticulously weighed and utilized for subsequent extraction procedures. The extraction of Ficus benjamina L was conducted employing a straightforward maceration technique. In this method, 400 grams of the crude plant powder were combined with 1.5 liters of methanol solvent in an extraction vessel, which was then left at room temperature (25°C) for a duration of three

days with periodic agitation. After the allotted extraction period, the solvent was separated from the plant material through filtration using Whatman filter paper[6]. Subsequently, the solvent extract was concentrated utilizing a rotary evaporator, yielding a concentrated extract. This concentrated extract was then transferred into sample vials for further analysis and experimentation.

In Vivo Anti-inflammatory study:

Chemicals and instruments:

The experiment utilized chemicals sourced from the Faculty of Pharmacy Bahauddin Zakariya University, Multan, Pakistan. Carrageenan was acquired from the Department of Pharmacology, while the reference standard was generously provided as a gift sample from the Department of Pharmaceutics at the same university. For the in-vivo assessments, a Plethysmometer (UGO Basile 7140 Italy) was employed to measure activity.

Animal selection:

The in-vivo evaluation of anti-inflammatory activity involved healthy female Wister Albino rats weighing between 120-200 grams, procured from the Animal House within the Faculty of Pharmacy Department at Bahauddin Zakariya University, Multan, Punjab, Pakistan. The rats were accommodated in a regulated environment, where humidity was upheld at 50-60% and temperature was maintained within the range of 20-25°C, guaranteeing a pathogen-free setting. Throughout the experimentation period, the animals were individually housed in separate cages within the animal facility, adhering to standard laboratory protocols. They were provided with a balanced diet and unrestricted access to water ad libitum to ensure their well-being.

Procedure:

All animals (n=5) were subjected to overnight fasting, with access restricted solely to water ad libitum, ensuring consistent hydration levels. Subsequently, the rats were divided into five groups, with each individual meticulously weighed using a precision balance. Paw volume measurements were acquired for each rat utilizing a Plethysmometer. Group 1 served as the control and received a normal saline treatment.

Group 2 was administered the standard treatment of Diclofenac Sodium. Groups 3, 4, and 5 were subjected to doses of 100mg/kg, 200mg/kg, and 300mg/kg, respectively, of the methanolic extract of the plant.Following group allocation and treatments, 0.1ml of 1% Carrageenan solution was injected into the left hind paw of each albino rat, with the time of injection duly recorded. The extract was orally administered to the rats 30 minutes subsequent to Carrageenan induction. Subsequent paw volume readings were recorded at various time intervals (0 hour, 1 hour, 2 hours, and 3 hours) for each albino rat.The percentage inhibition of each group was determined using the following formula:

$$\% \text{ inhibition} = (Vc - Vt / Vc) \times 100.$$

Statistical analysis:

The data provided in this study are presented as Mean ± SD calculated from individual observations. Statistical analysis for the in-vivo anti-inflammatory activity was performed using one-way ANOVA, with significance indicated by $P < 0.05$.

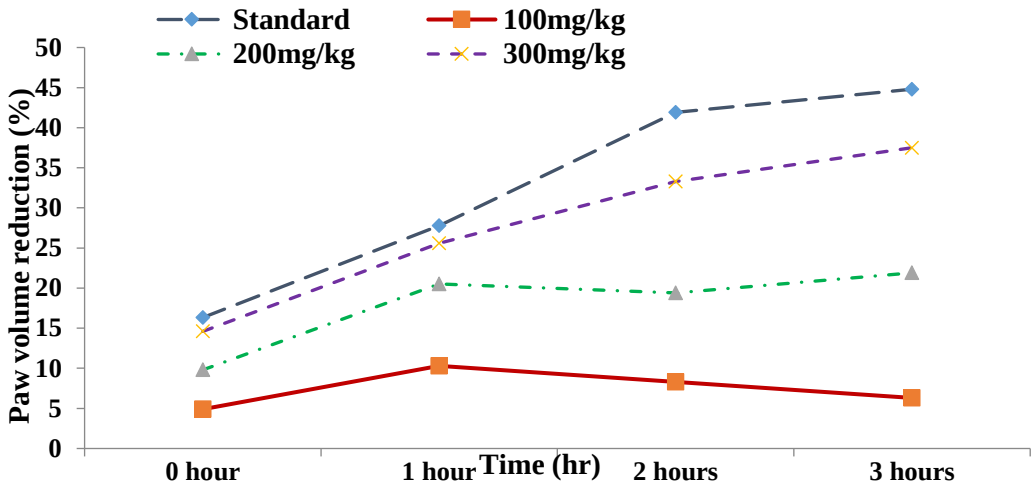

Figure 1. Paw volume reduction of *Ficus benjamina L* at different concentration of doses was compared with the effect of Standard Diclofenac Sodium.

Results:

The in-vivo anti-inflammatory activity was assessed by administering doses of 100mg/kg, 200mg/kg, and 300mg/kg of the methanolic extract from Ficus benjamina L, in comparison with the standard diclofenac sodium at a dose of 10mg/kg (Figure 1). The results indicated maximal activity at 14.63%, 25.64%, 33.33%, and 37.50% respectively, observed at a dose of 300 mg/kg across different time intervals. In comparison, diclofenac sodium exhibited activities of 16%, 27.77%, 41.93%, and 44.82% at a dose of 10 mg/kg (Table 2 and Figure 3). Notably, the methanolic extracts of Ficus benjamina L demonstrated remarkable anti-inflammatory properties, particularly at a dose of 300mg/kg (Table 1 and Figure 2).

Table 1: Anti- inflammatory activity by carrageenan induced paw edema. (Mean ± SD) (n=5)

Drug & Doses (mg/kg)	Carrageen induced edema (Volume in ml)			
	Time (h)			
	0 hour	1 hour	2 hours	3 hours
Control	0.49±0.026	0.54±0.030	0.58±0.015	0.62±0.026
Diclofenac	0.41±0.020**	0.39±0.017**	0.36±0.017**	0.32±0.020**
100mg/kg	0.39±0.017**	0.35±0.010**	0.33±0.020**	0.30±0.017**
200mg/kg	0.37±0.026**	0.31±0.017**	0.29±0.026**	0.25±0.017**
300mg/kg	0.35±0.017**	0.29±0.026**	0.24±0.017**	0.20±0.010**

* P<0.05 with control

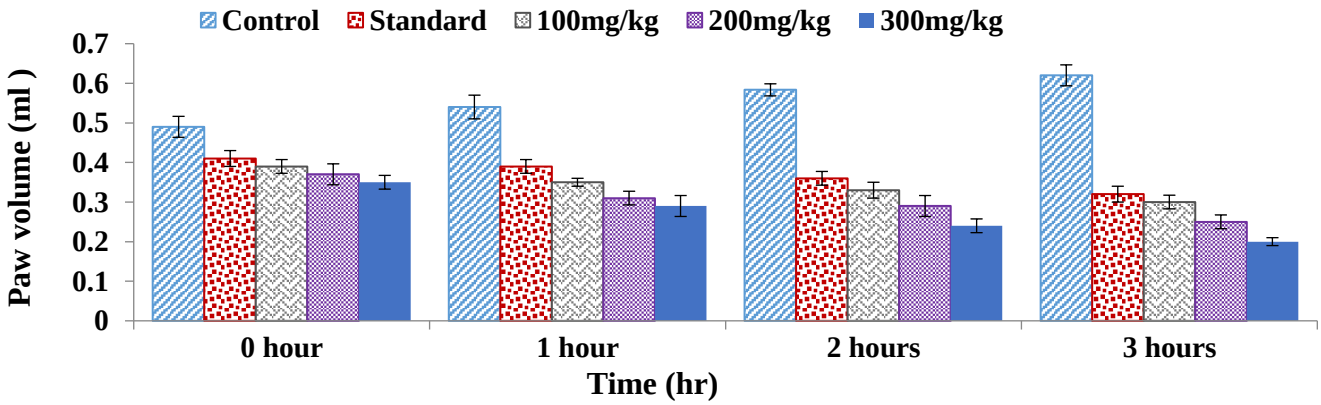

Figure 2. Graph showing results of Control, standard and methanolic extract of *Ficus benjamina L* on the rat paw volume.

Table 2. Percentage inhibition of inflammation, induced by carrageenan**.**

	Paw volume reduction (%)			
	Time (h)			
Groups	**0 hour**	**1 hour**	**2 hours**	**3 hours**
Diclofenac sodium (10mg/kg)	16%	27.77%	41.93%	44.82%
Dose 100mg/kg	5%	10.25%	8.33%	6.25%
Dose 200mg/kg	9.75%	20.51%	19.44%	21.87%
Dose 300mg/kg	14.63%	25.64%	33.33%	37.50%

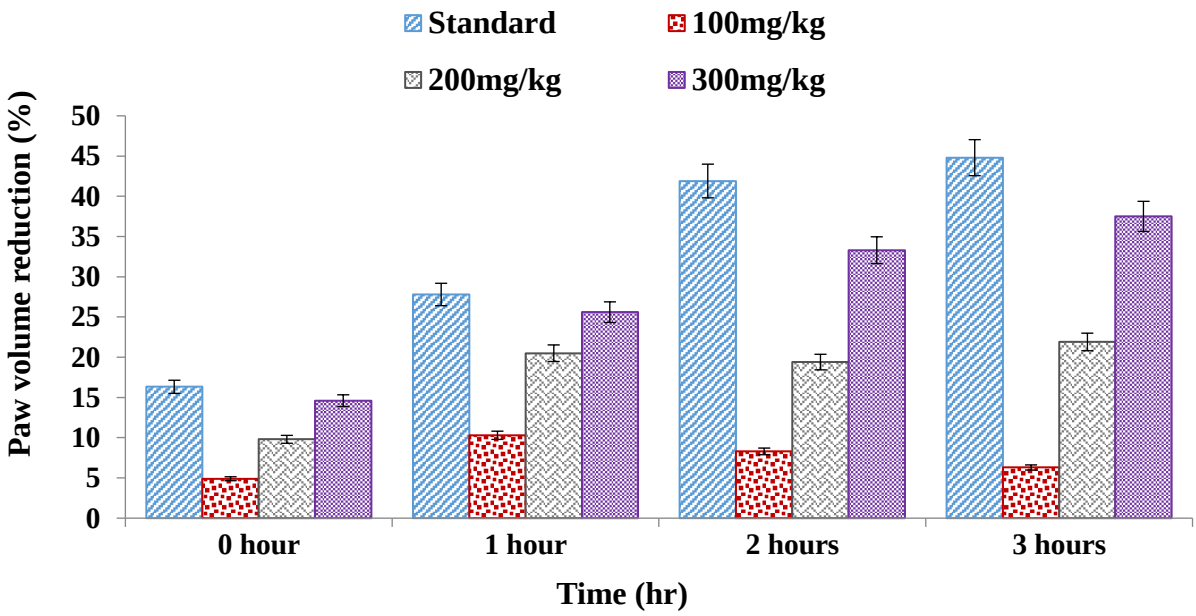

Figure 3. The graphical presentation showing the percentage inhibition of Rat hind paw healed from swelling due to Carrageenan injection**.**

Discussion:

The research aimed to assess the anti-inflammatory effects of methanolic extracts from Ficus benjamina L using albino rats in a Carrageenan-induced paw edema model. Results showed a dose-dependent decrease in paw edema volume with Ficus benjamina L extract treatment, with the most notable reduction observed at 300mg/kg. Comparatively, the extract displayed similar or enhanced efficacy in reducing inflammation compared to the standard anti-inflammatory drug, diclofenac sodium[7]. This dose-dependent response underscores the potent anti-inflammatory properties of Ficus benjamina L extract, aligning with its traditional medicinal uses[8]. Its superiority over diclofenac sodium at certain doses suggests potential as a natural alternative to synthetic drugs[9, 10]. However, further investigation is needed to understand its mode of action and potential synergies with other compounds. These findings hold promise for developing new anti-inflammatory agents from natural sources, with Ficus benjamina L extract warranting further pharmacological and clinical exploration. As interest in natural remedies grows, it could offer a valuable option for inflammatory disorders[11, 12]. The study also emphasizes the importance of blending traditional knowledge with modern scientific methods in drug discovery[13]. Yet, limitations, such as the use of animal models and focus on acute inflammation, underscore the need for caution when applying findings to clinical settings. Future research should explore its effects on chronic inflammatory conditions and validate its safety and efficacy in humans.

Conclusion:

In conclusion, this study affirms the robust anti-inflammatory properties of methanolic extracts derived from Ficus benjamina L, as evidenced by the dose-dependent decrease in paw edema volume in albino rats. These results substantiate the long-standing traditional medicinal applications of Ficus benjamina L and propose its viability as a natural substitute for synthetic anti-inflammatory medications. Future investigations should focus on validating its effectiveness and safety in human subjects, as well as elucidating its underlying mechanisms. In essence, Ficus benjamina L extract emerges as a promising therapeutic option for managing inflammatory ailments.

Conflict of interests:

The authors declare that we have no conflect of interests in this article.

Acknowledgements:

I am very thankful to the Faculty of Pharmacy, department of Pharmaceutical chemistry of Bahauddin Zakariya University Multan, Punjab Pakistan, for their help with lab facility and necessary requirement for this activity.

References:

1. Azeem, M., et al., *An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review.* Polymer Bulletin, 2023. 80(1): p. 241-262.
2. Abid, H.M.U., et al., *Wound-healing and antibacterial activity of the quercetin–4-formyl phenyl boronic acid complex against bacterial pathogens of diabetic foot ulcer.* ACS omega, 2022. 7(28): p. 24415-24422.
3. Ahmad, M., et al., *Expression characteristics and significant diagnostic and prognostic values of ANLN in human cancers.* International Journal of General Medicine, 2022: p. 1957-1972.
4. Azeem, M., et al., *Enhanced antibacterial and antioxidant properties of chitosan-quercetin complex containing polycaprolactone microspheres for the treatment of gastroenteritis: An in-vitro and in-vivo analysis.* Materials Today Communications, 2022. 31: p. 103780.
5. Hanif, M., et al., *Improved anti-inflammatory effect of curcumin by designing self-emulsifying drug delivery system.* Drug Development and Industrial Pharmacy, 2021. 47(9): p. 1432-1438.
6. Aleem, A., et al., *Microbial analysis of selected brands of whitening creams.* Saudi J. Med. Pharm. Sci, 2020. 6: p. 178-182.
7. Azeem, M., et al., *Design, synthesis, spectroscopic characterization, in-vitro antibacterial evaluation and in-silico analysis of polycaprolactone containing chitosan-quercetin microspheres.* Journal of Biomolecular Structure and Dynamics, 2023. 41(15): p. 7084-7103.
8. Muhammad, D., et al., *Antihepatotoxicity of Tagetes erecta (Mexican marigold) against indomethacin-induced Antihepatotoxicity in Sprague Dawley rats.* 2020.
9. Abid, S., et al., *Unlocking the potential of phenyl boronic acid functionalized-quercetin nanoparticles: Advancing antibacterial efficacy and diabetic wound healing.* Heliyon, 2024. 10(1).
10. Akhlaq, M., et al., *Antioxidant, Immunomodulatory and Fungicidal Potential of Different Extracts of Raphanus Raphanistrum L. Var. Caudatus.* Journal of Health and Rehabilitation Research, 2024. 4(1): p. 646-651.
11. Usman Abid, H.M., et al., *Exploring the Potent Combination of Quercetin–Boronic Acid, Epalrestat, and Urea Containing Nanoethosomal Keratolytic Gel for the Treatment of Diabetic Neuropathic Pain: In Vitro and In Vivo Studies.* Molecular Pharmaceutics, 2023. 20(7): p. 3623-3631.
12. Abid, H.M.U., et al., *NAVIGATING THE VASCULAR FRONTIER: EXPLORING QUERCETIN-BASED DRUG DELIVERY STRATEGIES IN CARDIOVASCULAR THERAPY.* Journal of Population Therapeutics and Clinical Pharmacology, 2024. 31(1): p. 1702-1719.

13. Abid, U., et al., *Fabrication and characterization of novel semi-IPN hydrogels based on xanthan gum and polyvinyl pyrrolidone-co-poly (2-acrylamido-2-methyl propane sulfonic acid) for the controlled delivery of venlafaxine.* Polymer-Plastics Technology and Materials, 2022. 61(6): p. 577-592.