INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Article

Antiarthritic Potential of Ethanolic Extract of Verbennaofficinalisin Rheumatoid Arthritis in Rats

Aakash Bhawalkar, Dr. Yashraj Yadav*

Swami Vivekanand College of Pharmacy, Indore M.P.

ARTICLE INFO

Published: 11 June 2025
Keywords:
Verbena officinalis, anti-arthritic activity, Wistar rats, turpentine oil, joint edema, inflammation, Asprin, herbal medicine, arthritis model
DOI:
10.5281/zenodo.15641173

ABSTRACT

Arthritis is a chronic inflammatory condition characterized by joint pain, swelling, and functional impairment. Verbena officinalis (Vervain) is traditionally used for its anti-inflammatory and analgesic properties. This study evaluates the anti-arthritic potential of Verbena officinalis root extract in Wistar rats using the turpentine oil-induced joint edema model.Arthritis was induced in Wistar rats by intra-articular injection of turpentine oil. Animals were divided into control, standard (Asprin-treated), and test groups receiving Verbena officinalis root extract at different doses. Joint edema, paw thickness, and behavioral parameters were assessed. Verbena officinalis root extract significantly reduced joint swelling and improved mobility compared to the control group. A dose-dependent inhibition of inflammatory markers was observed, with effects comparable to Aspirin. Histopathological analysis further confirmed reduced joint inflammation in treated groups. The findings suggest that Verbena officinalis root extract exhibits significant anti-arthritic activity, potentially through its anti-inflammatory and analgesic effects. Further studies are warranted to elucidate its mechanisms and therapeutic potential for arthritis management.

INTRODUCTION

Arthritis refers to a broad spectrum of inflammatory joint diseases characterized by pain, stiffness, swelling, and decreased mobility. It is a common condition affecting people of all ages, with a higher prevalence observed in older adults. Arthritis involves the degeneration or inflammation of the synovium, cartilage, and bone within the joints, which can lead to functional disability if untreated. There are over 100 different types of arthritis, but the most common are rheumatoid arthritis (RA), osteoarthritis (OA), gout, and psoriatic arthritis. [1-3].Osteoarthritis, the most common form of arthritis, is a degenerative joint disease primarily affecting the cartilage that cushions the joints. Over time, the

***Corresponding Author:** Dr. Yashraj Yadav
Address: *Swami Vivekanand College of Pharmacy, Indore M.P.*
Email ✉: yashrajyadavr@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

cartilage breaks down, leading to pain, stiffness, and inflammation. OA usually affects weight-bearing joints such as the knees, hips, and spine. It is often associated with aging, but factors such as joint injury, genetics, and obesity can accelerate its progression.[4-6] Rheumatoid arthritis is a chronic autoimmune disease where the body's immune system mistakenly attacks healthy joint tissues, primarily the synovium, leading to inflammation and eventual joint destruction. RA commonly affects small joints, such as those in the hands, wrists, and feet, and can also affect other organs in the body. The exact cause of RA is unclear, but genetic and environmental factors, such as smoking and infections, are believed to play a role.[7-13] Verbena officinalis (commonly known as vervain) has been traditionally used in various cultures for its medicinal properties, including its anti-inflammatory, analgesic, and antirheumatic effects. While its precise mechanisms of action in arthritis are still under investigation, several potential reasons explain why Verbena officinalis may be effective in treating arthritis: One of the primary features of arthritis, especially rheumatoid arthritis (RA), is persistent inflammation in the joints. Verbena officinalis contains several bioactive compounds, such as flavonoids, terpenoids, phenolic acids, and saponins, that are known to have potent anti-inflammatory effects. These compounds can modulate inflammatory pathways in the body, reducing the production of pro-inflammatory cytokines and mediators. Verbena officinalis has been shown to suppress the production of key inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). These cytokines play central roles in the inflammatory process and are involved in the pathogenesis of arthritis.The nuclear factor kappa B (NF-κB) pathway is a critical signaling pathway involved in the activation of inflammation. Compounds from Verbena officinalis may inhibit NF-κB, thereby reducing the activation of pro-inflammatory genes.[14-15]Oxidative stress is a significant contributor to the progression of arthritis, leading to tissue damage and joint degradation. Inflammatory processes in arthritis generate reactive oxygen species (ROS), which damage joint tissues (cartilage, synovium, and bone).Animal studies and in vitro experiments have shown that Verbena officinalis can significantly reduce joint swelling and the infiltration of inflammatory cells into the synovium. This anti-inflammatory effect likely arises from the combined actions of its antioxidant, anti-inflammatory, and immune-modulating components.[16] Verbena is a more or less hairy herb, growing up to 90 centimeters in height, erect, but decumbent at the base. Leaves are 5 to 10 centimeters long, variously lobed and narrowed to the base; the lower ones are stalked, pinnatifid or coarsely toothed, more or less hairy, and usually hoary on the nerves beneath; these upper ones are without stalks and 3-lobed. Flowers are small, 4 to 6 millimeters long, without stalks and borne on dense, bracteate heads which elongate as the fruit ripens. The calyx is twice as long as the bracts and half as long as the corolla tube, minutely 5-toothed, and glandular- hairy. The corolla is blue or lilac, and hairy, with spreading limb; the lobes are subquadrate, with a hairy throat. Fruit is dry, ultimately spreading into four 1-seeded nutlets which are oblong and dorsally smooth, their under faces covered with minute, white flaking cells. A weed in waste places in and about towns, at low and medium altitudes, only in the provinces of cagayan, Isabela and Nueva Viscaya Provinces in Luzon. Introduced, cosmopolitan in sub temperate

and subtropical regions. The most characteristic chemical constituents of vervain are the iridoid glycosides Verbenalin and hastatoside. Also prominent is the caffeic acid glycoside verbascoside, which is found in a number of other medicinal plants. Flavonoids, such as luteolin 7-diglucuronide have been isolated in vervain as have rsolic acid, sterols, and several related triterpenes. iridoid glycosides, sterols, and littorachalcone have been found in related verbena species (Verbenalitoralis and brasiliensis). [17]

2. MATERIAL AND METHOD

- **Procurement of the plant parts**

The plant material was collected from local area. At the time of collection whole plant including roots, stem and fruits were collected.

- **Treatment of the plant part**

The roots at the beginning went through a process called stabilization, whereby the roots were steamed in 60% pure ethanol. Subsequently, the roots had the thorns removed with special tools, and then were put in a well-ventilated place in the shade for drying. Drying usually lasted 7-15 days (depending on the weather). The thorns of the fruits were removed with a special brush; the fruits were then peeled and the peel removed was also placed for drying under the same conditions as the roots. They were then subjected to a stabilization process. They were subsequently placed in the sun to dry 1–2 days prior to being used for the extraction. The stabilization process for natural entities which are intended to be used in an extraction process after being dried, involves the following: placement of the respective compounds in a form of a very thin layer on the top of a sieve tray; a very fine droplet spraying of the herbal substance with pure ethanol follows, so that the whole exterior surface of it is wetted. The ethanol is then left to dry. This process is the most suitable natural method of elimination of any type of fungi or bacteria which can affect the herbal entities and prevent its decay until it is taken for the extraction

- **Preparation of the ethonalic extracts**

- The extraction process involved:

A. Extraction:

Sample of the root was crushed into pieces and then milled into course powder. A total of 400 g of the course powder was extracted using the Soxhlet apparatus. Briefly, 200 g of root powder was sequentially extracted with 1.5 L of 70% ethanol three times after every 4 hours. The extracts was transferred into a separating funnel and diluted with 100 mL of distilled water. It was then successively partitioned three times each with 500 mL petroleum ether, followed by 500 mL each of ethyl acetate three times. Each fraction was separately combined and dried in vacuum at 40° C. The aqueous fraction was also freeze dried. The dried solids were labeled.

- **Phytochemical tests**

For the phytochemical investigation, 1% of ethanolic extract of each extract was used following methods for phytochemical screenings were applied on extract.

a) Carbohydrates

Fehling's test; 1 ml Fehling's A solution and 1 ml Fehling's B solution were mixed and boiled it for 1 minute. Now the equal volume of test solution

was added to above mixture. The solution was heated in water bath for 5-10 minutes. First yellow and then brick red precipitate was obtained. This confirmed the presence of sugar.

b) Flavone Glycosides

Molisch test; 2 ml of test solution is placed in test tube. 2 drops of Molisch reagent (a solution of α-napthol in 95% ethanol) was added. The solution was poured slowly into a tube which contained 2 ml of sulphuric acid then two layers were formed. There was formation of purple product at the interface of the two layers.

c) Flavonoids

On addition of an increasing amount of sodium hydroxide, the extract was shown yellow coloration; this was decolorized after addition of dilute hydrochloric acid.

d) Steroids and Triterpenoids

Salkowski Test; Treat the extract with few drops of concentrated sulphuric acid, red color at lower layer indicates presence of steroid and formation of yellow color lower layer indicates presence of Triterpenoids.

3. Pharmacological Method

3.1 Animals

Wistar albino rats, weighing 260–270 g, were obtained from the animal house of the Department of Pharmacology of the Swami Vivekanand College of Pharmacy, Indore, India. Animals were housed at four per cage, allow free access to water and food, and maintain under constant temperature (23±1 0C) and humidity (60±10%) under a 12-h light/dark cycle (light on 07.30–19.30 h). Animal treatment and maintenance was conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. #85-23 revised 1985).

3.2 Experimental design

A total number of 25 rats were divided into following groups:

- Group I: control (Normal Saline, 2ml/kg)
- Group II: standard (Aspirin 100mg/kg ip)
- Group III: Test (Aqueous extract of roots of *VerbennaOfficinalis*100 mg/kg ip)
- Group IV: Test (Aqueous extract of roots of*VerbennaOfficinalis*200 mg/kg ip)
- Group V: Negative Control

3.3 Procedure

Each group was treated with the respective treatment as follows:

• Group 1 (Control): The rats received normal saline (0.9% NaCl) to maintain the baseline for comparison.

• Group 2: The rats received an oral dose of 100 mg/kg of the aqueous extract of Verbena officinalis.

• Group 3: The rats received an oral dose of 200 mg/kg of the aqueous extract of Verbena officinalis.

- Group 4: The rats received an oral dose of 100 mg/kg of Aspirin, a known anti-inflammatory agent, as a positive control.
- Group 5: Negative Control

All treatments were administered ip to ensure the proper absorption of the substances in the gastrointestinal tract.The rats were observed throughout the experiment for any adverse effects or abnormalities. Inflammation was induced in the rats using a suitable model (such as joint swelling or paw edema).The joint diameter (or paw volume) was measured at predetermined time intervals, for example, every 1 hour or every 2 hours over a 6-hour period. The measurement of joint diameter served as an indicator of inflammation.

3.4 Turpentine oil induced joint edema in rats:

The animals were allowed free access to water. Animals were randomly divided into 4 groups of 5 rats each. Group I served as control and received normal saline (2 mL/kg/p.o.) and Group II served as standard drug treated group and received the standard drug, aspirin (100 mg/kg; ip). While, Group III, IV received aqueous extract of roots of VerbennaOfficinalis (100 and 200 mg/kg; ip), respectively, Aqueous extract and its fractions were dissolved or suspended in distilled water. Acute non-immunological inflammatory joint edema was induced by injecting 0.02 mL turpentine oil in synovial cavity of right knee joint 30 min after drug administration. Joint diameter was measured at hourly interval for 6 hours using digital verniercalliper. The percentage inhibition of edema was calculated using following formula (Dhage et al., 2013):

Percentage inhibition of edema = (1-Vt)/Vc x 100

Where, Vt and Vc are the joint diameter of treated and control rats.

3.5 Statically analysis:

The data were expressed as mean ± SEM. Results were analyzed statically by Way ANOVA followed by Dunnet's TEST using prime of Biostatistics, Version 9. The difference was considered significant if $p<0.05$.

4. RESULTS and

4.1 Acute Toxicity:

The LD 50 of the extract was found to be 2000 mg/kg. (As per literature survey)

Table 4.1 Indicating presence of various phytochemical constituents.

S. No.	Test	Positive/ Negative
1.	Carbohydrate	+
2.	Alkaloids	+
3.	Terpenoids	-
4.	Flavone Glycoside	+
5.	Phenolic Compound	+
6.	Flavonoids	+
7.	Saponins	-
8.	Sterols	+

Note:-(+) = Present or (-)= Absent

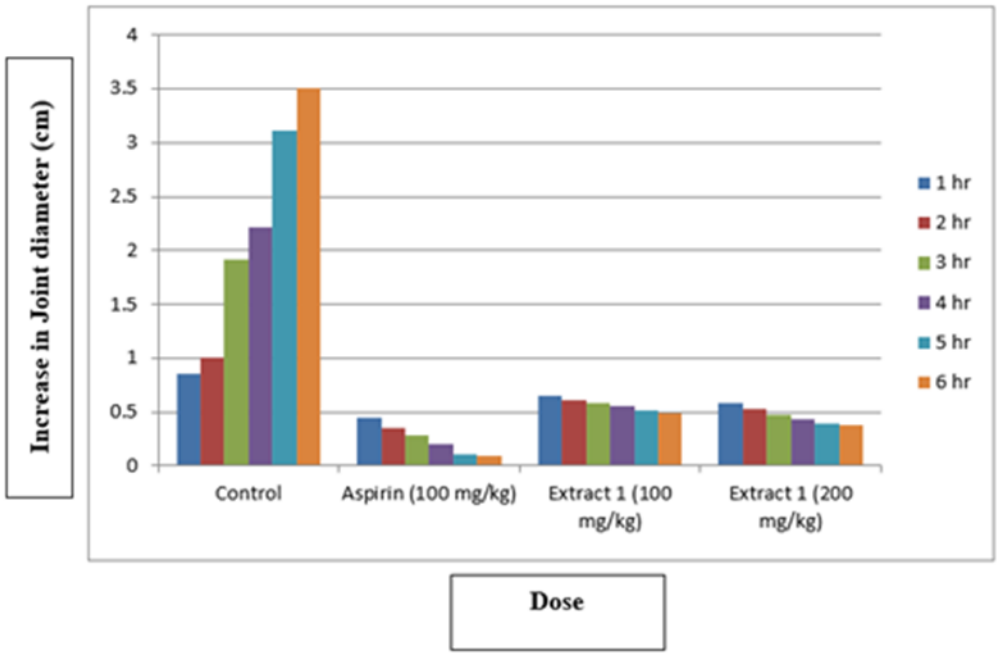
4.2 Anti-arthritic Activity:

4.2.1. Turpentine oil induced joint edema model:

Table 4.2 Effect of aqueous extract of roots of *Verbenna Officinalis* for Turpentine oil induced joint edema model.

Treatment	Increase in Joint diameter (cm)					
	1 hr	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
Control	0.86±0.03	1.01±0.12	1.91±0.23	2.21±0.19	3.11±0.34	3.51±0.35
Aspirin (100 mg/kg)	0.45±0.02*	0.35±0.17*	0.29±0.25*	0.20±0.10*	0.11±0.24*	0.09±0.10*
Extract 1 (100 mg/kg ip)	0.65±0.08	0.61±0.14	0.59±0.19	0.55±0.17	0.51±0.31	0.49±0.32
Extract 2 (200 mg/kg ip)	0.58±0.02***	0.53±0.15***	0.48±0.20***	0.43±0.14***	0.40±0.29***	0.38±0.33***
All value are given in mean±SEM, *P < 0.05, **P < 0.01 as compare with the control group (one way ANOVA followed by Dunnett's test).						

Graph 4.1 Effect of aqueous extract of roots of *Verbenna Officinalis* for Turpentine oil induced joint edema model.



5.2 Treatment Results:

The study assessed the increase in joint diameter (indicative of inflammation) over 6 hours in control, standard (aspirin), and two doses of the plant extract. Key observations include:

1. **Control Group:** Showed a continuous and significant increase in joint diameter over time, reaching a maximum of **4.02±0.35 cm** at 6 hours.

2. **Aspirin (100 mg/kg):** Demonstrated a significant and consistent reduction in joint swelling, with the diameter decreasing to **0.09±0.10 cm** at 6 hours.

3. **Extract 1 (100 mg/kg):** Showed moderate reduction in joint diameter, reaching **0.49±0.32 cm** at 6 hours.

4. **Extract 2 (200 mg/kg):** Displayed better anti-inflammatory activity compared to the lower dose, reducing joint diameter to **0.38±0.33 cm** at 6 hours, though it was not as effective as aspirin.

5.3 CONCLUSION:

Based on the discussion above, it is clear that *V. Officinalis* has demonstrated a significant anti-arthritic effect in experimental studies. Although the exact mechanism by which *V. Officinalis* alleviates arthritis remains unidentified, its positive effects on rheumatoid arthritis (RA) may be linked to the presence of alkaloids (such as Verbenalin and Hastatoside), previously found in *V. Officinalis*, as well as phenols and flavonoids identified in the current study. In conclusion, this research provides pharmacological evidence supporting the traditional use of *V. Officinalis* in treating and managing painful arthritic inflammatory conditions. Based on these findings, additional in-depth studies are needed to better understand the precise mechanism of action of *V. Officinalis*, assess levels of pro-inflammatory cytokines, isolate active compounds, and conduct cellular characterization, all of which could solidify *V. Officinalis* as a potentially safer disease-modifying agent for RA treatment.The study aimed to evaluate the anti-arthritic potential of *Verbena officinalis*, with a focus on its phytochemical properties and their role in reducing joint inflammation. Phytochemical screening of the plant extract revealed the presence of bioactive compounds, including carbohydrates, alkaloids, flavonoid glycosides, phenolic compounds, flavonoids, and sterols, which are known for their anti-inflammatory and antioxidant activities. Terpenoids and saponins were absent from the extract.The anti-inflammatory effects were tested by measuring the increase in joint diameter (a marker of inflammation) over 6 hours in different treatment groups: a control group, a standard (aspirin), and two doses of the plant extract (100 mg/kg and 200 mg/kg). The control group showed significant swelling, reaching a maximum joint diameter of 4.02±0.35 cm. The aspirin treatment effectively reduced inflammation, with joint diameter decreasing to 0.09±0.10 cm. Both doses of the plant extract showed moderate reductions in joint swelling, with the higher dose (200 mg/kg) being more effective than the lower dose (100 mg/kg), though not as potent as aspirin. The study concluded that *Verbena officinalis* exhibits significant anti-arthritic activity, likely due to its bioactive compounds such as flavonoids and phenolic compounds, which are known for their anti-inflammatory properties. While the plant extract demonstrated dose-dependent efficacy in reducing joint swelling, it was less effective than aspirin. These findings suggest that *Verbena officinalis* could be a promising natural remedy for arthritis, but further research is needed to isolate the active compounds, understand their mechanisms of action, and assess their effectiveness in clinical trials.

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HOW TO CITE: Aakash Bhawalkar, Dr. Yashraj Yadav*, Antiarthritic Potential of Ethanolic Extract of Verbennaofficinalisin Rheumatoid Arthritis in Rats, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 6, 2187-2195. https://doi.org/10.5281/zenodo.15641173