AUCTORES
Globalize your Research

Journal of Clinical and Laboratory Research
Donatus O. Anele *

Open Access

Research Article

Anti-Inflammatory Potential of Medicago Sativa (Alfalfa) Ethanol Leaf Extract Against Xylene-Induced Ear Edema in Wistar Rats

Donatus O. Anele [1*], Franklin E. Igwe [2], Michael C. Orih [3], Victor Okoroukwu [1], Ezekwesiri Ekeke [1], Dike Onwubiko [4]

[1]Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Gregory University, Uturu, Abia State, Nigeria.

[2]Department of Anatomical Pathology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

[3]Department of Chemical Pathology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

[4]Department of Chemical Pathology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

***Corresponding Author:** Donatus O. Anele., Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Gregory University, Uturu, Abia State, Nigeria.

Received Date: 06 July 2026 | **Accepted Date:** 17 July 2026 | **Published Date:** 24 July 2026

Citation: Donatus O. Anele, Franklin E. Igwe., Michael C. Orih., Victor Okoroukwu, Ezekwesiri Ekeke., et al, (2026), Anti-Inflammatory Potential of Medicago Sativa (Alfalfa) Ethanol Leaf Extract Against Xylene-Induced Ear Edema in Wistar Rats, *Journal of Clinical and Laboratory Research*, 9(3); **DOI:**10.31579/2768-0487/210

Abstract

Background: This research investigates the anti-inflammatory potential of ethanol leaf extract from Medicago sativa (Alfalfa) against xylene-induced ear edema in Wistar rats.

Objectives: The study aims to assess the inhibitory effect of Medicago sativa ethanol leaf extract on acute ear edema induced by xylene in comparison to a standard anti-inflammatory drug, dexamethasone.

Methods: Fresh leaves of Medicago sativa were collected from specific locations in Imo State, Nigeria, identified, and authenticated by a taxonomist. A sample specimen was deposited in the University herbarium for reference. The leaves were air-dried, pulverized, and extracted with 80% ethanol. The extract was concentrated, dried, and reconstituted in distilled water to obtain desired doses. Thirty Swiss rats were used, divided into five groups. The rats were acclimatized, fasted, and subjected to xylene-induced ear edema. Different groups received Medicago sativa leaf extract at varying doses, and a control group received distilled water. Dexamethasone was administered as a standard drug.

Results: Significant differences ($p < 0.05$) in edema sizes were observed between treated and untreated ears. Ethanol extract of M. sativa showed notable inhibitory activity, comparable to dexamethasone. The anti-inflammatory effect increased with higher doses of M. sativa, with 78% inhibition at 600 mg/kg. Statistical analysis confirmed significant differences ($p < 0.05$) compared to the control group.

Conclusion: The study demonstrates the potential anti-inflammatory effect of Medicago sativa ethanol leaf extract against xylene-induced ear edema in Wistar rats. The findings suggest that the extract exhibits inhibitory activity comparable to the standard drug, dexamethasone.

Key words: medicago sativa; ethanol leaf extract; anti-inflammatory; xylene-induced ear edema; wistar rats; dexamethasone; inhibition

Introduction

Inflammation is a complex biological response to harmful stimuli, such as pathogens, damaged cells, or irritants, aimed at removing the cause of cell injury and initiating tissue repair [1,2]. However, dysregulated or chronic inflammation can lead to various diseases, including autoimmune disorders, cardiovascular diseases, and cancer. Medicinal plants have long been recognized for their anti-inflammatory properties, making them promising candidates for the development of therapeutic agents. Medicago sativa, commonly known as alfalfa, is a perennial flowering

plant belonging to the legume family Fabaceae. Traditionally used in folk medicine for its diverse pharmacological activities, alfalfa has gained attention for its potential anti-inflammatory effects. The anti-inflammatory properties of Medicago sativa are attributed to its rich phytochemical composition. Flavonoids, such as quercetin and kaempferol, have been reported to possess anti-inflammatory effects by modulating inflammatory pathways [3]. The ethanol leaf extract of Medicago sativa, in particular, has demonstrated significant anti-inflammatory potential in vitro [2]. Studies have explored the anti-inflammatory effects of Medicago sativa in different experimental models. Extracts from various parts of the plant, including leaves and seeds, have demonstrated inhibition of pro-inflammatory cytokines and mediators, emphasizing its potential as an anti-inflammatory agent [4,5]. Xylene-induced ear edema is a well-established model for evaluating the anti-inflammatory activity of natural compounds. Xylene exposure triggers a robust inflammatory response characterized by increased vascular permeability and edema formation, making it a suitable model to assess the efficacy of anti-inflammatory agents [6,7]. The potential mechanisms underlying the anti-inflammatory effects of Medicago sativa include the inhibition of pro-inflammatory cytokines (e.g., TNF-α, IL-1β), modulation of oxidative stress, and interference with signalling pathways such as NF-κB and MAPK [1,8]. The current research aims to investigate the anti-inflammatory potential of Medicago sativa ethanol leaf extract against xylene-induced ear edema in Wistar rats. Xylene, a solvent widely used in various industries, induces acute inflammation, making it a suitable model for assessing anti-inflammatory agents.

Materials And Methods

Plant Collection and Identification

Fresh leaves of Medicago sativa (Alfalfa) were collected from farms at Obinze and Eziobodo, both in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified and authenticated by Mr. Ibe Ndukwe, a taxonomist in the Department of Forestry, College of Environmental Sciences, Michael Okpara University of Agriculture, Umudike, Abia State and a sample specimen MOUAU/ZEB/21/009 was deposited in the University herbarium for reference.

Extraction of Plant Materials

The leaves were sliced into pieces, air-dried at room temperature and pulverized into powder using a Warring commercial blender. Eight hundred grams (800 g) of the coarse powder of M. sativa leaves were weighed by a sensitive digital weighing balance. The powder was soaked in a flask containing 80% ethanol (2.5 L w/v) and then placed on a shaker with occasional shaking for 48 hours at room temperature. The mixture formed was filtered using Whatman (No.1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield of 30.45 g (4 % w/w) dry extract with a greenish colour. It was preserved in a refrigerator at 4°C for further analysis. The extract was later reconstituted in distilled water to give desired doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. The percentage yield was calculated using the formula of Ezirim et al. [9] as stated below:

$$\%\ \text{yield} = \frac{\textbf{Weight(g) of residue (dry extract)}}{\textbf{Weight(g) of powdered material grinded}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

Experimental Design

Thirty (30) Swiss rats of either sex were used in this study. The animals were sourced from Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka. They were kept in the animal house of the Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Abia State University, Uturu, Abia State. The animals were acclimatized for 14 days prior to the experiment. They were fed with standard diet (Ladokun feeds, Ibadan) and had access to water ad libitum. They were maintained under standard conditions of humidity, temperature and 12 hours light and 12 hours darkness cycle. The animals were used in accordance with the National Institute of Health guide for the care and use of Laboratory Animals [10].

The 30 Swiss rats were divided into 5 groups of 6 rats in each cage. The animals were fasted for 24 hours prior to the experiment but allowed access to water ad libitum. Group 1 served as control and was administered with distilled water (20 mL/kg). M. sativa leaf extract (200 mg/kg, 400 mg/kg and 600 mg/kg, p.o) was given to groups 2, 3 and 4 respectively, while the standard drug, dexamethasone (4 mg/kg), was administered to group 5. One hour post drug administration, oedema was induced in each mouse by applying a drop of xylene at the inner right ear. Three hours afterwards, the animals were sacrificed under halothane anaesthesia and both ears were cut off to equal size and weighed. Inflammation was taken as mean difference between the right and left ear for each group according to the method of Jumping et al. [11].

Results

All the ears of treated rats exhibited topical oedema induced by xylene. There was a significant difference in sizes of oedema ($p < 0.05$) between the treated right and the untreated left ears in all extracts tested. On the effect of the extract on acute oedema, ethanol extract of M. sativa exhibited more inhibitory activity (Table 1 and & Figure.1). The anti-inflammatory activity is comparable to dexametasone, the standard drug which was also significant ($p<0.01$).

Treatment Groups	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% Inhibition
Control	0.073±0.01	0.031±0.00	0.042±0.00	–
200 mg/kg of *M. sativa*	0.050±0.00	0.029±0.00	0.021±0.00	50[a]
400 mg/kg of *M. sativa*	0.038±0.00	0.025±0.00	0.013±0.00	70[b]
600 mg/kg of *M. sativa*	0.032±0.01	0.021±0.00	0.010±0.00	78[b]
4 mg/kg of Dexamethasone	0.029±0.00	0.020±0.00	0.009±0.00	80[b]

Table 1: The effect of ethanol leaf extract of M. sativa on xylene-induced ear oedema in rats.

One-way ANOVA + Dunnett's post hoc test (n=6). a P<0.05; b P<0.01 compared to control.

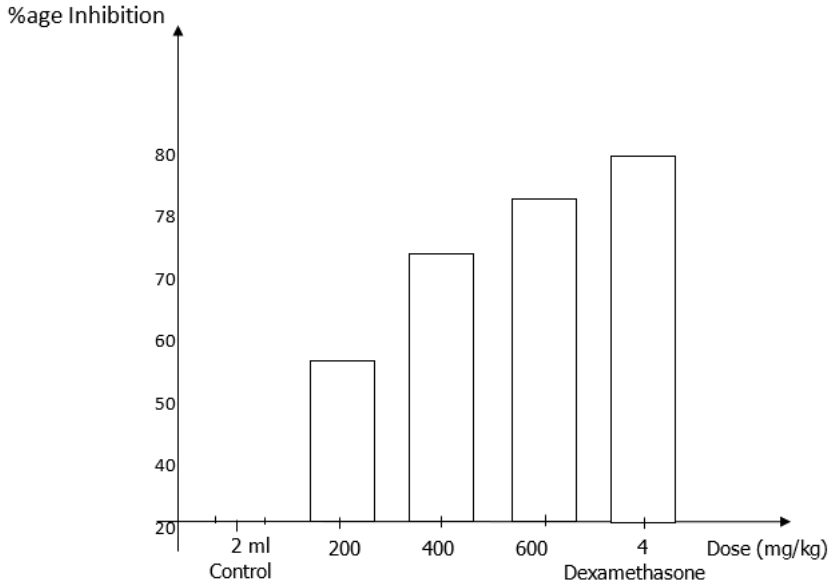
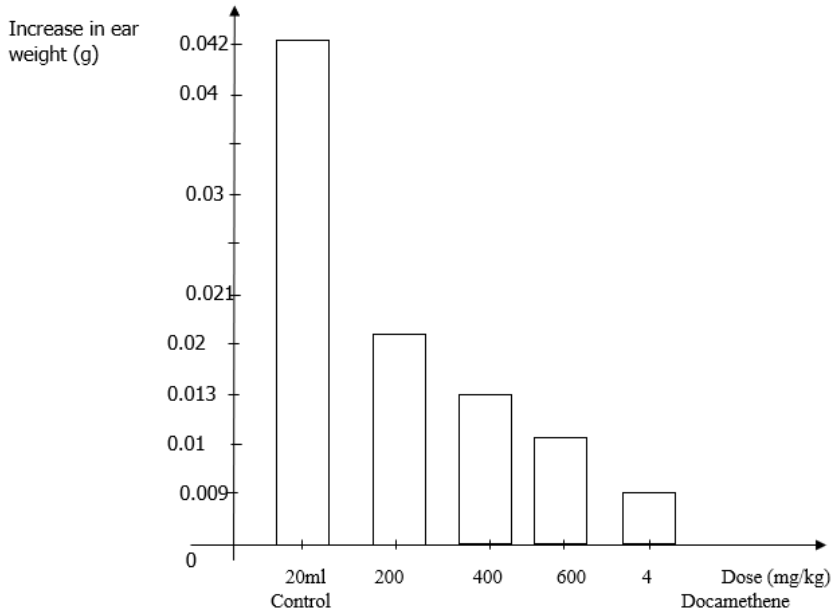


Figure 1: Bar Chart of ethanol leaf extract of *M. sativa* extract and xylene–induced ear edema.



Discussion

Xylene is known to cause irritation in mouse ear which leads to accumulation of fluid and edema, characteristic of acute inflammatory response; suppression of this response may indicate antiphlogistic effect [12]. The leaf extract of Medicago sativa exhibited a significant inhibition of ear edema caused by Xylene. This activity suggests the inhibition of phospholipase A2 which is involved in the patho-physiology of inflammation due to xylene [13]. The anti-inflammatory activity of dexamethasone may be due to the presence of flavonoids, saponins, tannins, tarpenes and alkaloids. The inhibition was dose-dependent and may have been initiated by the presence of one or some of the bioactive compounds contained in the extract.

In Rheumatoid arthritis, flavonoids were reported to inhibit the release of chemical mediators through the histamine while serotonin reduce the symptoms. These were thought to be mediated through decreased monocyte infiltration and fibroblast proliferation, blocked TNF-∝ and inhibited COX pathway [14]. This plant leaf extract might have followed this mode of action pathway in inflammation.

The mechanism of anti-inflammatory activity for the revealed phytoconstituents in M. sativa had been reported in literature. One of such was through the inhibition of NF–KB activation and downregulation of the expression of inflammatory enzyme markers such as 5–COX, COX–2 and MMP–9 [15].

Conclusion

The results of this study demonstrated a significant inhibitory effect on acute edema in rats treated with M. sativa extract, as evidenced by a notable reduction in the size of edema compared to the control group. The inhibitory activity was dose-dependent, with higher doses (400 mg/kg and 600 mg/kg) exhibiting greater efficacy. The findings of this research suggest that the ethanol leaf extract of M. sativa possesses anti-

inflammatory properties comparable to the standard drug, dexamethasone, as indicated by the significant reduction in ear edema. These results support the traditional use of Medicago sativa in herbal medicine for its anti-inflammatory potential. The study provides valuable insights into the pharmacological actions of M. sativa and lays the groundwork for further exploration of its bioactive compounds responsible for the observed anti-inflammatory effects. Future research may delve into elucidating the molecular mechanisms underlying the anti-inflammatory activity of M. sativa and conducting clinical trials to validate its therapeutic potential in managing inflammatory conditions. Overall, the study contributes to the growing body of evidence supporting the medicinal properties of Medicago sativa, emphasizing its potential as a natural remedy for inflammatory disorders.

References

1. Wu, Y., Li, L., Wen, X., Xie, R., & Zhang, Y. (2022). Anti-inflammatory and antioxidant effects of alfalfa (Medicago sativa L.) leaf extract on lipopolysaccharide-induced inflammatory response in RAW264.7 cells. *Journal of Food Biochemistry*, 46(2), e13442.
2. Zhao, Y., Liu, Y., Lan, X., Wang, C., Zhang, X., & Xu, Y. (2021). Anti-inflammatory and anti-arthritic effects of polysaccharides extracted from alfalfa (Medicago sativa L.) leaves. *International Journal of Biological Macromolecules*, 167, 882–890.
3. Sultana, S., Ahmad, S., & Khan, N. (2019). Quercetin prevents inflammation and collagen accumulation in ovalbumin-induced lung inflammation in Wistar rats. *Inflammation*, 42(1), 258–270.
4. Li, X., Wang, J., Zhang, J., Zhang, X., Zhang, Y., & Hu, X. (2019). Protective effects of alfalfa saponin extract against acute ethanol-induced liver injury in mice. *Pharmaceutical Biology*, 57(1), 186–192.
5. Wang, X., Wang, Y., Zhu, L., & Fan, R. (2020). Isolation, structure, and bioactivities of the polysaccharides from alfalfa (Medicago sativa L.). *International Journal of Biological Macromolecules*, 147, 477–483.
6. Ribeiro, A. R., Diniz, P. B., Pinheiro, M. S., Albuquerque-Júnior, R. L. C., Thomazzi, S. M., et al. (2018). Anti-inflammatory and anti-ulcer activities of carnosol, a diterpene present in Rosmarinus officinalis L. *Journal of Natural Medicines*, 72(2), 282–291.
7. Shin, S. H., Lee, J. Y., Jang, D. S., Kim, J. S., Kim, J. C., et al. (2017). Anti-inflammatory effects of an ethanol extract of Ecklonia cava on lipopolysaccharide-induced inflammation in vitro and in vivo. *BMC Complementary and Alternative Medicine*, 17(1), 442.
8. Zhou, Y., Chen, J., Zhang, H., Chen, X., & Sun, H. (2020). Antioxidant, anti-inflammatory and anti-apoptotic effects of aqueous extracts of alfalfa on acute exercise-induced liver injury in mice. *Molecular Medicine Reports*, 22(6), 4961–4970.
9. Ezirim, E.O., Uche, C.L., Abali, I.O., Iwuoha, C.E., Chika-Igwenyi, N.M., et al. (2022). Therapeutic potential of Parkia biglobosa seed against potassium bromate-induced testicular toxicity. *International Journal of Research and Reports in Gynaecology* 5(3): 78- 89.
10. (2011). National Research Council. Guide for the Care and Use of Laboratory Animals, 8th ed. *The National Academies* Press: Washington, DC, USA,
11. Jumping, K., Yun, N., Wang, N., Liang, L., Zhi – Hong, H (2005) Analgesic and anti – inflammatory activities of total extract and individual functions of Chinese medicinal plant polyrrchasis lamellidens. *Bio. Pharm. Bull*., vol. 28: 176 – 180.
12. Akuodor, G.C., Idris-Usman, M., Ugwu, T.C., Akpan, J.L., Irogbeyi, L.A., et al. (2010). Ethanolic leaf extract of Verbena hastate produces antidiarrhoeal and gastrointestinal slowing effects in albino rats. *Journal of Medicinal Plants Research*, 4: 1624–1627.
13. Caughey GH. (2016). "Mast cell proteases as pharmacological targets". Pharmacological modulation of mast cells and basophils. *European Journal of Pharmacology*. 778: 44 -55.
14. Pahwa R, Goyal A, Bansal P and Jialal I (2021). "Chronic Inflammation". *National Institutes of Health – National Library of Medicine*. (https://pubmed.ncbi.nlm.nih.gov/29630225).
15. Firke, S.D., & Bari, S.B (2015). Synthesis, biological evaluation and docking study of maleimide derivatives bearing benzene sulfonamide as selective COX-2 inhibitors and anti-inflammatory agents. *Bio org. Med.Chem*., vol. 23 (17): 5273-5281.

DOI:10.31579/2768-0487/210