# Evaluation of the Anti-Tubercular Role of Nimbolide in Animal Model of Tuberculous Arthritis

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**Abstract**

**Objective:** To evaluate the effect of Nimbolide on tuberculous arthritis in mice.

**Material and Method:** 24 BALB/c mice were divided by simple random sampling into three groups with 8 animals in each group: normal control, diseased control, and treatment group. Tuberculous arthritis was induced by injecting 10mg of Mycobacterial tuberculosis H37Rv strain in 1 ml of normal saline into the tail vein of diseased control and treatment groups. Nimbolide was administered to the treatment group at the dose of 0.2 mg/kg for 4 weeks from the 5th to the 9th week. Mice were sacrificed under ether anesthesia after 9 weeks.

**Results:** Nimbolide increased body weight in mice affected by tuberculous arthritis ($p < 0.001$). Nimbolide improved the histopathological changes associated with tuberculous arthritis ($p < 0.001$).

**Conclusion:** Nimbolide has shown anti-tubercular arthritic activity in mice model of tuberculous arthritis. Nimbolide can be a possible agent for the treatment of tuberculosis and tuberculous arthritis.

**Keywords:** Nimbolide, Tubercular arthritis

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## Introduction

Mycobacterium tuberculosis is the causative agent of Tuberculosis (TB), which is an important cause of death. Almost 10 million people are affected by TB and each year 1.5 million people die from TB worldwide.[1]. TB is more common in low-income countries and it further increases poverty. Pakistan ranks 5th among the countries with a high TB burden.[2] TB mainly targets the lungs, but other extra-pulmonary tissues and organs are also involved. Other sites are the lymph nodes, pleura, urogenital tract, and musculoskeletal system. In the musculoskeletal system, TB mainly affects the spine. Other than the spine, TB may affect any bone or joint.[3] TB causes structural damage and deformity leading to impairment of joints and bones. Treatment options are medical and surgical therapy. After surgical treatment, there are high chances of recurrence, which can be prevented by long-term drug therapy.[4]

There are different drug regimens available for the treatment of TB. However these treatment regimens are becoming ineffective owing to the development of drug resistance[5]. The drugs available for the cure of TB are costly, associated with many side effects, and less efficacious. There is an increasing need to explore medicinal plants as a cure for TB. Medications derived from various plants are used for different diseases in many countries. Plant-based medications are cost-effective and easily available. There is also less chance of resistance associated with medicines obtained from plants. Thus, anti-TB drugs derived from plants can be an alternative to classic anti-TB drugs.[6]

Azadirachta indica commonly known as the Neem tree is found in India, Bangladesh, Pakistan, Nepal, Africa, and other tropics. Neem Leaves, bark, and seeds have shown important anti-inflammatory, anti-bacterial, anti-tubercular, anti-protozoal, anti-malarial, anti-arthritic, insect repellent, anti-cancer and immune-modula-

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tory effects.[7] Neem has been used for TB treatment previously.[8]

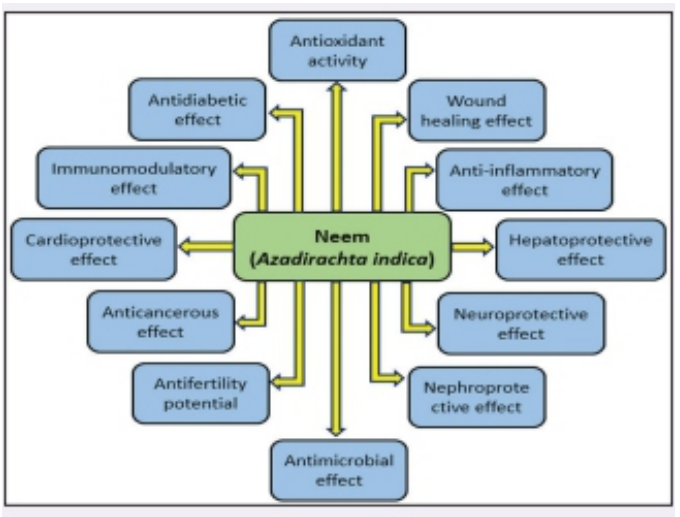


**Fig-1:** *Picture showing different effects of neem*[9]

Neem is enriched with limonoids. Nimbolide is the limonoid which is isolated from the leaves of neem. It possesses anti-oxidant, anti-cancer, and anti-inflammatory effects.[10,11] It is also shown to have antimicrobial, antimalarial, and anti-HIV activity.[12] Previously, Nimbolide has demonstrated anti-arthritic activity in rheumatoid arthritis induced by freund's adjuvant[13]. This study is designed to look for the anti-tubercular activity of Nimbolide in tubercular arthritis.

## Material and Method

It is an experimental study, performed at the Pharmacology Department, Postgraduate Medical Institute (PGMI), Lahore after ethical approval from institutional review board vide letter no. 00-13-A-2023 dated 17-05-2023. The 24 healthy BALB/c mice, 7-9 weeks of age, and weighing 9-12 grams were obtained from the PGMI, Lahore and confined in the animal house of PGMI. The animals were familiarized to 25±2°C temperature, 45-65% humidity, and kept in a light/dark cycle of 12h, under optimal conditions of hygiene. Animals had ad libitum access to rat chow and water. 24 BALB/c mice were divided by simple random sampling into three groups: normal control, diseased control, and treatment group. On day 1, 10mg of Mycobacterial tuberculosis H37Rv strain in 1 ml of normal saline was inoculated into the tail vein of diseased control and treatment groups.[14] 1 ml of saline was injected as a vehicle in the tail vein of the normal control group. Nimbolide was given to the treatment group at the dose of 0.2mg/kg[15] per oral dissolved in 1 ml of 0.1 % DMSO (Dimethyl Sulfoxide) for 4 weeks from the 5$^{th}$ to the 9$^{th}$ week. Mice from the normal and diseased control groups were given 1 ml of 0.1% DMSO per oral from the 5$^{th}$ till the 9$^{th}$ week for 4 weeks. Mice were sacrificed under ether anesthesia after 9 weeks[16]. Each mouse was weighed with a precision balance every week. After sacrificing mice, ankle joints were dislocated and preserved in 10% formalin. Then they were placed in a decalcifying solution for decalcification. Paraffin blocks were made. Then thin tissue slices were cut with the help of a microtome and placed on a glass slide. Slides were stained with hematoxylin and eosin.[3] A histopathologist examined the slides in a blinded manner and looked for inflammation, granuloma formation, and destruction of cartilage. The slides were scored from 0 to 3, where a score of 0 was given when there were no pathological changes. Scores 1 to 3 were given to mild, moderate, and severe changes. To determine statistical significance One-way ANOVA was applied. Post-hoc Tukey's test was applied to determine group mean differences. p-value ≤ 0.05 was reflected as statistically significant.

## Results

Tuberculosis is associated with weight loss. Mice in the diseased group exhibited a (p-value <0.001) statistically significant decrease in body weight in comparison to the control group after disease induction to the last day of the study. Mice from the treatment group exhibited a (p-value < 0.001) increase in body weight significantly on days 45 and 60 as compared to the diseased group, as presented in figure no. 2. Tuberculosis is associated with inflammation. There was a significant increase in inflammation in disease control (2.8±0.2) in comparison to normal control as presented in figure no. 7. Mice that were treated with Nimbolide showed a statistically significant reduction in inflammation (1.7 ± 0.4 vs 2.8±0.2) in comparison to mice in the diseased control group as presented in figure no. 3 and 9. It shows the anti-inflammatory potential of Nimbolide and advocates that Nimbolide can be used as an anti-inflammatory agent. Granulomas are a hallmark of TB infection. There was a significant increase in granuloma formation in diseased control (2.7 ± 0.2) in comparison to normal control as presented in figure no. 8. Mice who were given treatment with Nimbolide showed a statistically significant decrease in granuloma formation (1.1± 0.3 vs 2.7 ± 0.2) in comparison to mice in the diseased control group as presented in figure no 4. It shows the anti-tubercular potential of Nimbolide and proposes that Nimbo-

lide can be used as an anti-tubercular agent. Tubercular arthritis is associated with the destruction of articular cartilage. There was a significant destruction of cartilage in diseased control (2.2 ± 0.3) as compared to normal control. Mice who were given treatment with Nimbolide showed a decrease in cartilage destruction which was statistically significant (1.2± 0.4 vs 2.2 ± 0.3) in comparison to mice in the diseased control group as presented in figure no 5. These findings show the anti-arthritic potential of Nimbolide and suggests that Nimbolide can be used as an anti-arthritic agent in tuberculous arthritis.

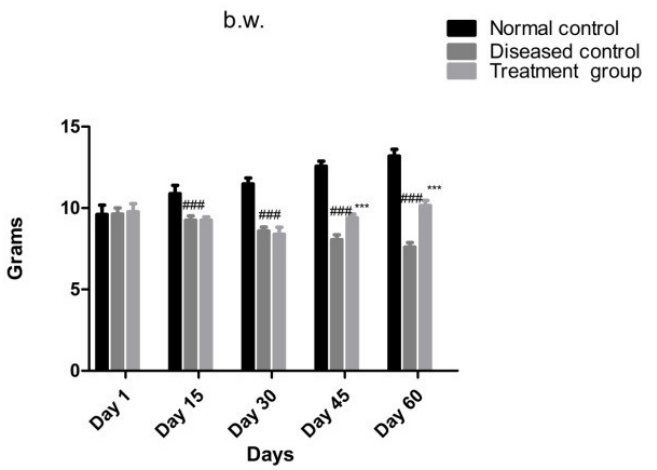


**Fig-2:** *Representation of the mean ± SD of body weight graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

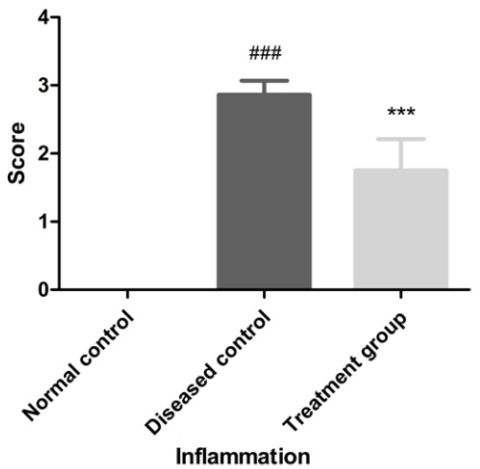


**Fig-3:** *Representation of the mean±SD of inflammation graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

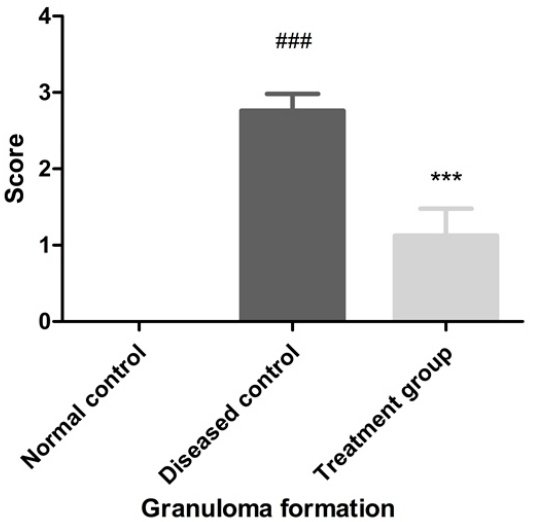


**Fig-4:** *Representation of the mean±SD of granuloma formation graphically in all groups (n = 8). ### shows p-value <0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

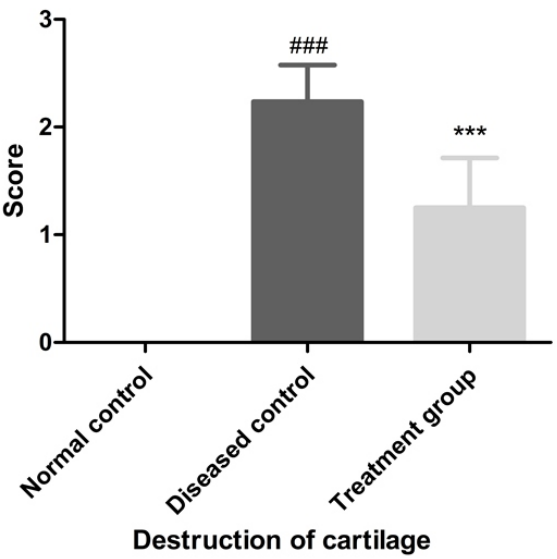


**Fig-5:** *Representation of the mean±SD of destruction of cartilage graphically in all groups (n = 8). ### shows p-value < 0.001 and shows a difference as compared to the control group significantly. *** shows p-value < 0.001 shows a difference as compared to the diseased group significantly.*

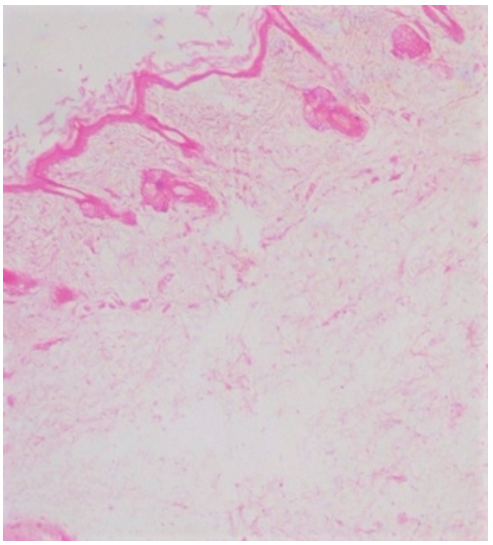

**Fig-6:** *Photomicrograph (ankle joint) displaying unremarkable inflammation (H & E; 4x) (Normal control)*

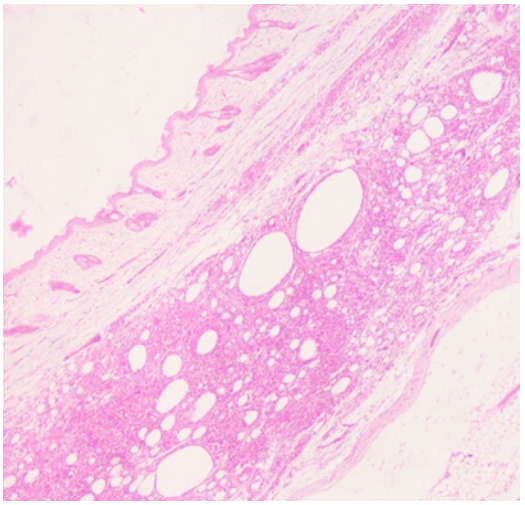

**Fig-7:** *Photomicrograph (ankle joint) displaying intense inflammation (H & E; 4x) (Diseased control)*

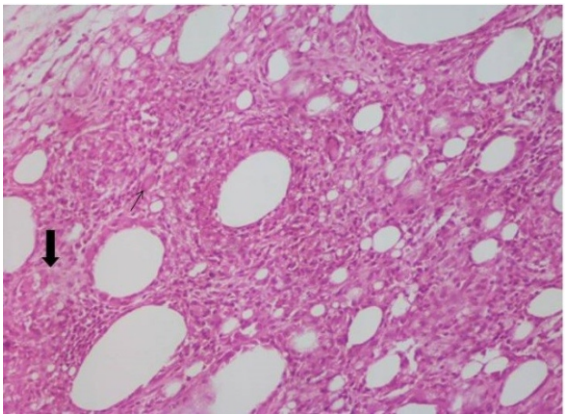

**Fig-8:** *Photomicrograph (ankle joint) displaying granuloma formation along with giant cell (H & E; 20x) (Diseased control)*

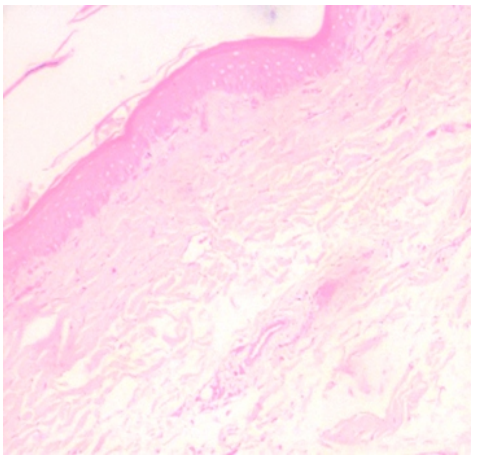

**Fig-9:** *Photomicrograph (ankle joint) displaying unremarkable inflammation (H & E; 4x) (Treatment group)*

## Discussion

Currently different drug regimens are used for the treatment of tuberculosis. However, there is an increased incidence of therapeutic failure due to emergence of anti-tubercular drug resistance.[17] Different medicinal plants are reported to have anti-tubercular potential and can be used as an adjunct drug to standard treatment for TB. Azadirachta indica (Neem) has proven anti-fungal, anti-bacterial, anti-viral, and anti-parasitic properties.[18] In a previous study, neem bark extract cured TB in animals infected with Mycobacterium tuberculosis H37Rv strain and it was non-toxic to the animals.[19] Nimbolide is obtained from neem leaves and it possesses anti-bacterial potential against many resistant micro-organisms.[20] In our present study mice with tuberculous arthritis showed a decrease in body weight while treatment with Nimbolide improved the body weight of mice. It has been reported previously that prolonged inflammation in TB leads to weight loss.[21] It signifies the anti-inflammatory and anti-tubercular roles of Nimbolide which is in line with a previous study where Nimbolide exhibited anti-inflammatory potential.[11] Histopathological examination of mice having tuberculous arthritis showed inflammation, granuloma formation, and cartilage destruction. These findings are in agreement with a previous study where TB infection was linked to inflammatory changes and granuloma formation.[3] Treatment with Nimbolide decreased inflammation and granuloma formation and it also restored the articular cartilage in diseased mice. These findings are in accordance with previous studies, where Nimbolide improved arthritic changes in arthritic rats.[22] These results propose the anti-tubercular and anti-arthritic roles of Nimbolide in tuberculous arthritis.

## Conclusion

Nimbolide has shown anti-tuberculous arthritic activity in mice model of tuberculous arthritis. It improved body weight in tuberculous arthritic rats and improved the histopathological changes associated with tuberculous arthritis. Nimbolide has the potential to be used for the treatment tuberculous arthritis.

**Conflict of Interest:** **None**

**Source of Funding:** **None**

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## Authors Contribution

**MI, SI:** Conceptualization of Project
**MI:** Data Collection
**SI:** Literature Search
**MN:** Statistical Analysis
**AHS:** Drafting, Revision
**RA:** Writing of Manuscript