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Biochemical And Hematological Impacts Of Thymosin Alpha 1 On Bone Tissue In Animal Models

Indu Bala[1], Pranav Kumar Prabhakar[1*]

[1, 1*]School of Allied Medical Sciences, Lovely Professional University, Punjab India-144411

***Correspondence:** Pranav Kumar Prabhakar
*Email: Pranav.16113@lpu.co.in

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Abstract

This study aimed to examine the effects of Thymosin Alpha 1 (Tα1) on the degree of arthritis and bone remodeling in animal models. The chronic autoimmune illness known as rheumatoid arthritis (RA) is marked by inflammation and joint damage, frequently with changes in bone metabolism. Tα1, an immune-modulatory peptide found naturally in the body, has demonstrated potential in reducing inflammatory reactions and accelerating tissue healing. The effects of Tα1 on arthritis progression and related bone alterations were evaluated in this study using a collagen-induced arthritis (CIA) model in wistar rats. Following treatment with Tα1 at three different doses (0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg), the animals were observed for signs and symptoms of arthritis, joint swelling. As indicated by a decrease in joint inflammation, our findings show that Tα1 therapy reduced the severity of arthritis. In arthritic joints, Tα1 therapy increased anti-inflammatory responses and inhibited the production of pro-inflammatory cytokines. Moreover, the injection of Tα1 resulted in an increase in osteoblast activity and a decrease in osteoclast-mediated bone resorption, hence enhancing bone quality and integrity. Tα1 may be a useful therapeutic agent for reducing the severity of arthritis and maintaining bone health in patients with RA, according to these data. Tα1's therapeutic potential in clinical settings will only be revealed through additional research into the molecular mechanisms behind its effects on arthritis and bone remodeling.

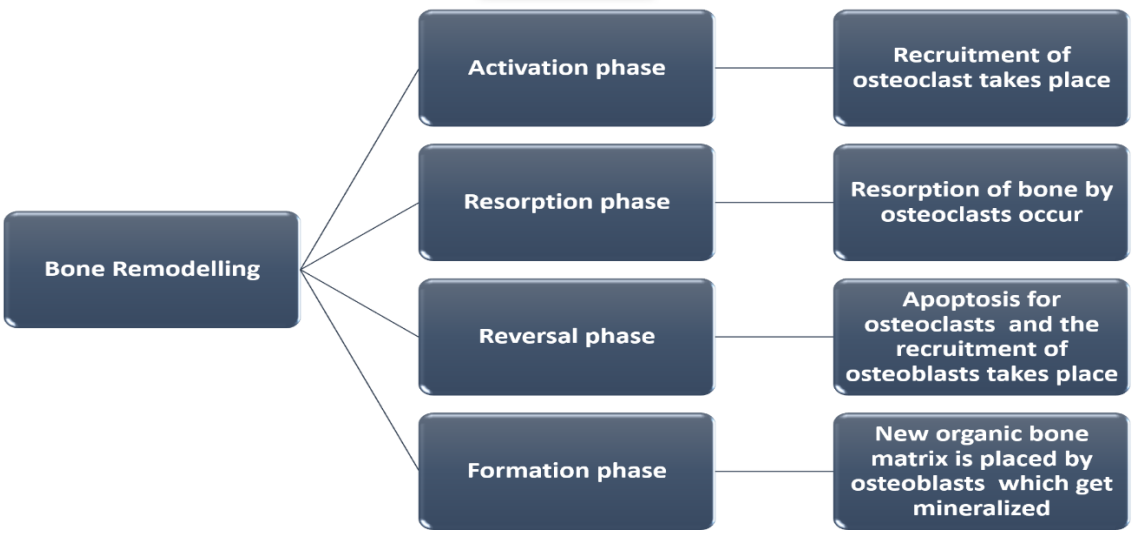
Keywords: CIA rats, rheumatoid arthritis, bone remodeling, inflammation, thymosin alpha-1

Introduction:

Muscles, bones, joints, tendons, ligaments, and other body parts can all be significantly impacted by musculoskeletal disorders, which include about 150 known illnesses and conditions. These ailments range from short-term ailments like sprains to long-term, incapacitating conditions like rheumatoid arthritis and osteoarthritis. In addition to causing discomfort and impeding mobility,

musculoskeletal issues can also impair a person's capacity for employment, social obligations, and mental health. Since 1990, lower back pain in particular has continuously been recognized as one of the major causes of disability worldwide, indicating the substantial impact these disorders have on societies all over the world. It is estimated that musculoskeletal disorders cause discomfort to 20% to 33% of the world's population (1). The age and diagnosis factors have a significant impact on this percentage. According to recent data from the USA, the prevalence of musculoskeletal illnesses in adults is on par with chronic respiratory and cardiovascular diseases (2). According to an analysis of data from the "World Health Organization's Study of Global Ageing and Adult Health (SAGE)", people with economically disadvantaged backgrounds are more likely to have arthritis than people in middle-class or wealthy countries (3).

Bone performs vital roles and is made up of cellular, vascular, and calcium compound structures: (a). It functions as a store for phosphate, magnesium, potassium, and bicarbonate and is essential for preserving calcium homeostasis. (b) Bone serves as a lever for muscle activity and gives soft tissues structural support. (c) It functions as our body's main location for hematopoiesis, the process that creates new blood cells (4). Bone needs to continually grow, repair, and break down (resorb) in order to stay healthy. All of these modifications to bones are collectively referred to as "remodeling". Remodeling is a result of osteoblast and osteoclast activity. There are four steps involved in bone remodeling (5):



A number of cytokines and hormones are essential for controlling bone remodeling. Bone loss, a defining feature of numerous skeletal illnesses such as osteoporosis and rheumatoid arthritis (RA), arises when the rate of new bone formation is not keeping up with the rate of bone destruction (6). Among the many systemic autoimmune diseases, RA stands out for its high frequency and enduring synovial inflammation, which deteriorates cartilage and joints. Research points to immune system dysfunctions, especially those involving pro-inflammatory reactions, as a factor in arthritis's chronicity. Th cells, in particular, are essential for both triggering and maintaining inflammation. Synthetic peptide thymosin alpha 1 reduces inflammation and increases the generation of different T cell subsets and natural killer cells, which in turn modifies immunological responses (7). Clinical research has investigated its potential impact on autoimmune illnesses including psoriatic arthritis, as well as diseases like cancer, severe sepsis, and hepatitis B and C (8). Researchers discovered that Ta1 may alter immune function and had conflicting effects on joint pain in individuals who had survived breast cancer (9). Significantly, patients with chronic inflammatory autoimmune illnesses have been found to have lower serum levels of Thymosin alpha 1, indicating the possible therapeutic value of this protein (10).

Experimental Model:

The Wistar strain albino male rats used in the experiment were 6-8 weeks old and were obtained from the Invivo Biosciences animal house facility located in Bengaluru, Karnataka 560091. They were allowed to acclimate to the animal house environment, maintained on a 12-hour light-dark cycle. Food and water were provided ad libitum. Eight rats per experimental group were divided into three categories: CIA control rats, normal control rats, and arthritic rats treated with 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg of Thymosin alpha-1. The animal ethics committee at the Invivo Biosciences animal house facility in Bengaluru, Karnataka, approved all experimental methods in advance.

CIA induction and dosage schedule:

The experimental design of the study included five groups of animal models, each subjected to different treatments to assess the role of Thymosin-α 1 in bone health. Group I served as the control group and received a vehicle treatment administered intraperitoneally (I.P.). Group II, the arthritis group, was induced with arthritis through the administration of Type II collagen on day 0 and lipopolysaccharide (LPS) on day 3. Group III, designated as the arthritis + Thymosin-α 1 (1 mg/kg) group, received the same arthritis-inducing agents as Group II, but with the addition of Thymosin-α 1 at a dose of 1 mg/kg administered I.P. on the 1st, 3rd, and 5th days. Group IV, the arthritis + Thymosin-α 1 (0.5 mg/kg) group, followed a similar protocol, receiving Thymosin-α 1 at a reduced dose of 0.5 mg/kg on the same days. Lastly, Group V, the arthritis + Thymosin-α 1 (0.25 mg/kg) group, also received the arthritis-inducing agents along with Thymosin-α 1 at a dose of 0.25 mg/kg I.P. on the 1st, 3rd, and 5th days. This setup allowed for the evaluation of the effects of different doses of Thymosin-α 1 on arthritis-induced bone changes.

After the dosage was finished, blood was drawn using the retro-orbital method, which allowed the serum to be separated. The laboratory study included the measurement of hematological indicators like RBC count, Differential count, and platelet counts in addition to the assessment of serum biochemistry markers like Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and ferritin.

Statistical analysis:

SEM was computed by averaging all of the data. Dunnett's multiple comparison tests were used to compare all of the treatment groups' parameters with those of the negative control group using a one-way ANOVA. A value of less than 0.05 was deemed statistically significant.

Result:

The rats, induced with arthritis, underwent a treatment regimen involving the administration of test substances on days 1, 3, and 5, with consistent timing of application. Throughout the entire study duration, meticulous daily monitoring was implemented to assess the overall health of all animals and to identify any discernible clinical changes. Swellings in the hind paw region were consistently observed in both the groups receiving treatment and the induced group. Upon the culmination of the 15-day study period, specifically on the 15th day, a thorough examination was conducted for all animals. This comprehensive assessment included an array of biochemical markers and haematological parameters. This approach ensured a comprehensive evaluation of the physiological responses and outcomes associated with the administered treatments and the induced arthritic conditions. An automated blood analyser was used to analyze the blood sample

for hematological parameters, and kit-based techniques were employed to complete all biochemical assays on the RoboniK semi-automated analyser.

Biochemical Parameters:

Serum ALT and AST:

On the 15th day of experimentation, serum ALT levels were examined, showing consistent levels across all groups, regardless of arthritis presence. The arthritic group exhibited ALT levels of 94.1±0.58 IU/ml, while normal rats showed levels of 94.1±0.80 IU/ml. Administration of different doses of Thymosin alpha-1 (0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg) did not yield significant changes in ALT levels in arthritic rats. Specifically, ALT levels were 94.8±0.77 IU/ml at 0.25 mg/kg, 95.9±0.81 IU/ml at 0.5 mg/kg, and 95.4±0.84 IU/ml at 1 mg/kg. None of the treatment groups exhibited notable differences compared to the arthritis control group (refer to Figure- 1 and 2). Similarly, serum AST levels remained consistent across all groups, with no significant changes observed following Thymosin alpha-1 administration, underscoring its hepatic safety profile.

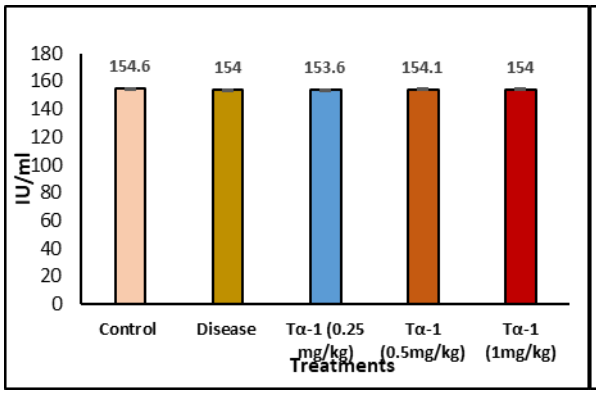


Fig. 1: Graphical representation of Serum ALT in all the five groups

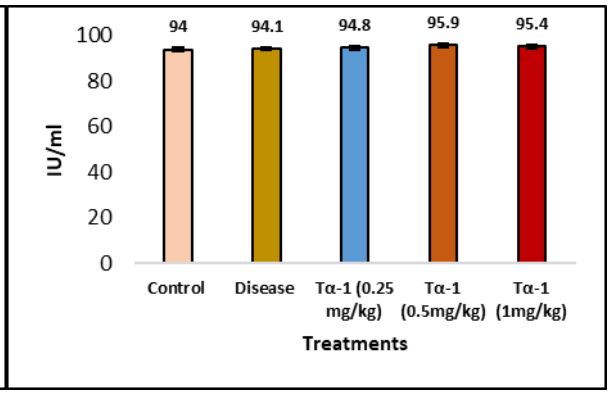


Fig. 2: Graphical representation of Serum levels AST levels in all the five groups

Serum Alkaline Phosphatase:

A significant rise in the serum levels of Alkaline Phosphatase was observed as arthritis progressed in arthritic control rats, with measurements on the 15th day reaching 211.4±0.95 U/L. Arthritic control rats exhibited a noteworthy difference in serum ALP levels compared to normal control rats, recording values at 112.6±0.41 U/L on the 15th day. Following the administration of Thymosin alpha-1 at varying doses (0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg), a marked decrease in serum levels of ALP in arthritic rats was evident on the 15th day. Specifically, the ALP levels were 197.5±3.75 U/L at the dosage of 0.25 mg/kg, 144.5±0.38 U/L at the dosage of 0.5 mg/kg, and 124.8±1.0 U/L at the dosage of 1 mg/kg. Recent investigations have further unveiled a noteworthy decrease in ALP levels within the low, mid, and high dose groups, as opposed to the arthritis control group **(Figure-3).** This observed reduction in ALP levels suggests a potential influence of the administered doses on the regulatory mechanisms of ALP, emphasizing the need for a more nuanced understanding of the relationship between Thymosin alpha-1 treatment and ALP dynamics in the context of RA. Further exploration of this phenomenon could contribute valuable insights into the complex interplay between Thymosin alpha-1 and the biochemical markers associated with RA pathology.

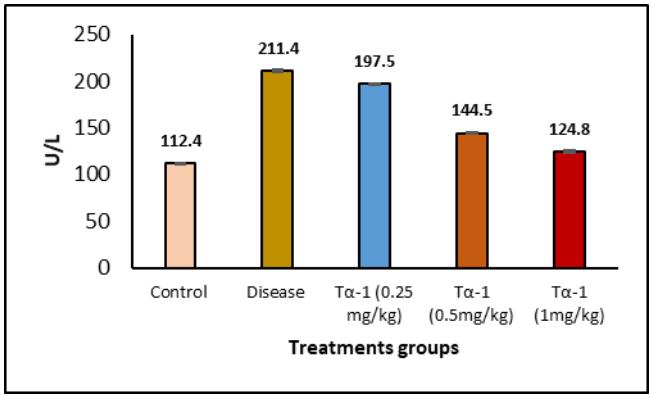


Fig. 3: Graphical representation of reduction in serum ALP levels post treatment with Thymosin alpha-1 comparing to disease group

Serum Ferritin Levels:

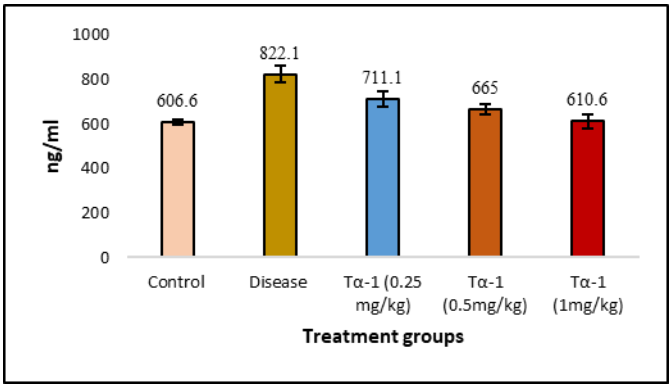


Fig. 4: Graphical representation of significant reduction in the levels of serum ferritin

A marked increase in serum Ferritin levels was evident as arthritis progressed in arthritic control rats, peaking at 822.1±36.14 ng/ml on the 15th day. Notably, arthritic control rats exhibited a substantial disparity in serum Ferritin levels compared to their non-arthritic counterparts, registering values of 606±12.9 ng/ml on the same day. Upon the administration of Thymosin alpha-1 at varying doses (0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg), a discernible reduction in serum Ferritin levels in arthritic rats became observable by the 15th day. Specifically, Ferritin levels recorded were 711.1±35.84 ng/ml at the 0.25 mg/kg dosage, 665±25.19 ng/ml at the 0.5 mg/kg dosage, and 610.1±33.65 ng/ml at the 1 mg/kg dosage. This observed decline suggests a potential therapeutic impact of Thymosin alpha-1 on the modulation of serum Ferritin levels in the context of arthritis progression. The findings emphasize the importance of investigating the influence of Thymosin alpha-1 on Ferritin as a crucial facet in comprehending its potential role in the management of arthritis. A substantial reduction in ferritin levels was evident across all treatment groups when compared to the arthritis control group **(Figure-4).**

Haematological Parameters:

Hematological parameters, including red blood cell (RBC) count, platelet count, neutrophil count, lymphocyte count, eosinophil count, and monocyte count, were assessed on the 15th day of the experiment. Notably, arthritic rats exhibited a significant decrease in RBC count compared to the control group, with a marked reduction observed following administration of Thymosin alpha-1 at varying doses (Figure-5). Platelet counts remained consistent across all groups, unaffected by

Thymosin alpha-1 treatment (Figure-6). Conversely, neutrophil count increased substantially in the arthritic group but decreased notably with Thymosin alpha-1 treatment (Figure-7). Lymphocyte count displayed a significant increase in the arthritic group, with reductions noted after Thymosin alpha-1 administration (Figure-8). Eosinophil and monocyte counts showed no significant changes with Thymosin alpha-1 treatment compared to the arthritic control group (Figure 9 and 10). These findings underscore the potential of Thymosin alpha-1 in mitigating arthritis progression.

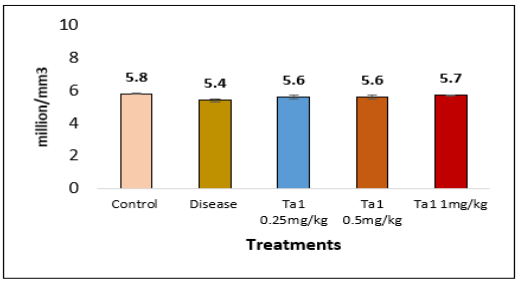


Fig.5: RBC count in all the groups

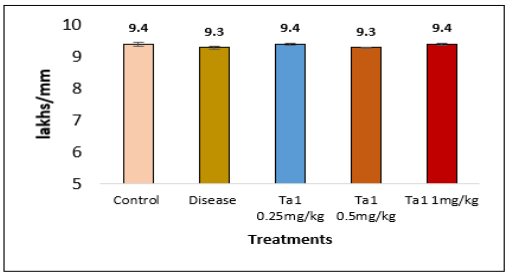


Fig.6: Platelets count in all the groups

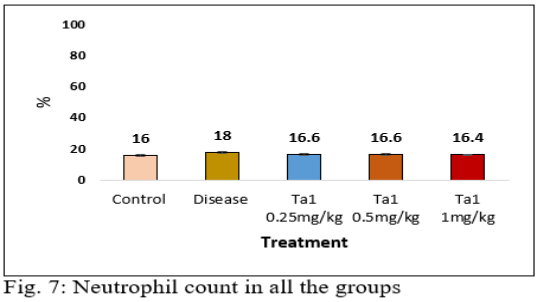


Fig. 7: Neutrophil count in all the groups

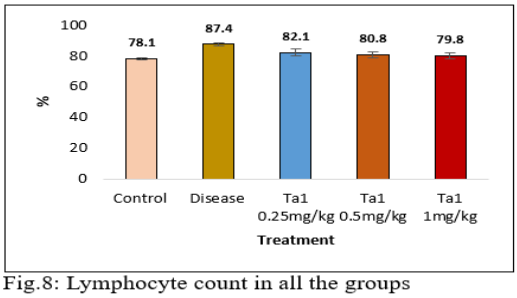


Fig.8: Lymphocyte count in all the groups

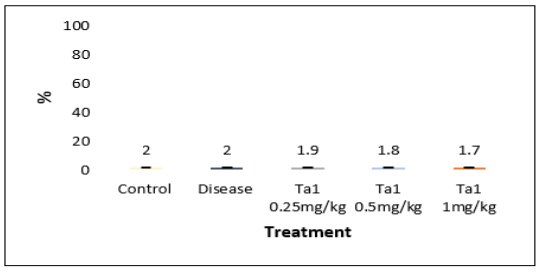


Fig.9: Eosinophil count in all the groups

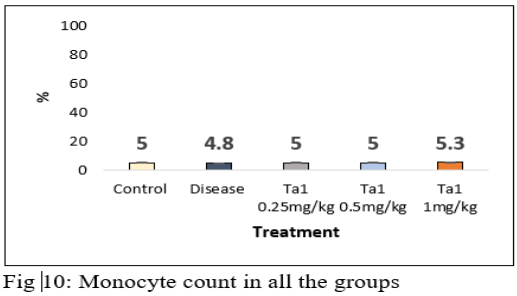


Fig |10: Monocyte count in all the groups

Discussion:

Lately, there has been much discussion about the investigation and adoption of biosimilars generic drugs that mimic biological DMARDs. There is evidence to support their efficacy, providing a noteworthy substitute to lower expenses, increase therapy alternatives, and lessen access discrepancies between developed and underdeveloped countries (11). The tendency of RA patients to have therapy failure even after trying every possible treatment option highlights the need for new medicines and a better understanding of the mechanisms underlying therapy toxicity and failure in non-remissive instances. Adverse effects and high costs present substantial obstacles to patients taking their drugs as prescribed (12). Originally derived from thymus tissue, thymosin alpha 1 is a synthetic peptide made of 28 amino acids that is presently synthesized (13). Research indicates that it may impact immune function and have different impacts on joint discomfort (9). The study findings indicate that following Thymosin alpha-1 treatment, significant improvements were noted in a number of measures, such as paw swelling, both biochemical and hematological parameters.

In 2010, Curtis JR. et al. conducted a research study and discovered that irregular ALT/AST levels emerged in 14-35% of patients starting DMARD therapy for RA or PsA. The risks were higher,

especially for those with PsA and those taking a combination of MTX (at least 10 mg/day) and LEF. These discoveries can guide the monitoring process for potential liver issuesin these patient groups (14). In earlier studies, many treatments approaches were associated with an increase in liver markers. In the current study we have not seen any significant difference in the levels of AST/ALT in all the groups. Thymosin alpha-1 did not exhibit any adverse effects on the liver markers ALT, as observed in those previous studies.

Increased levels of serum ALP are associated with inflammation in RA. In 1998, Niino-Nanke Y. et.al. conducted a study determining that out of 123 patients 37.4% of individuals with rheumatoid arthritis (RA) exhibited elevated serum alkaline phosphatase (ALP) values, measuring at 245.2 +/- 91.2 IU/L. These values were notably higher than those observed in osteoarthritis (OA) patients, averaging 192.3 +/- 45.2 IU/L ($P < 0.01$, RA vs. OA).The research findings in RA patients further confirmed a positive correlation between the increase in serum ALP levels and the activity of the disease (15). However, in the present study with the administration of Thymosin alpha-1 the levels of ALP were reduced in all the 3 groups at dosage (0.25mg/kg, 0.5mg/kg and 1mg/kg).

R. S. Rothwell. et al. also confirmed in their research study that in the context of acute rheumatoid disease, serum ferritin serves as an acute-phase reactant, reflecting the intensity of disease activity. Within the cohort of 15 patients, observations reveal notable declines in serumferritin levels that correspond with a decrease in disease activity, as evaluated through the Ritchie index and ESR (16). The similarity between our study and Rothwell et al.'s research strengthens the idea that serum ferritin is a reliable sign of how active rheumatoid disease is. This agreement not only supports the trustworthiness of using serum ferritin as an indicator but also shows that this link holds true in various research studies. This shared evidence adds what we know about how important serum ferritin is in understanding RA disease better, helping us grasp its clinical significance more fully. In current study also a substantial reduction in ferritin levels was evident across all treatment groups when compared to the arthritis control group. Overall, the present study data showed improvement in the paw, biochemical parameters and hematological parameters. Thymosin alpha-1 has demonstrated promising efficacy in reducing the pathophysiological manifestations of rheumatoid arthritis (RA), suggesting its potential as a superior therapeutic candidate for future RA management strategies. Further detailed analysis of mechanistic studies may offer a deeper understanding of its mode of action and enhance its therapeutic utility in RA treatment.

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