RESEARCH PAPER

Anti-Inflammatory Activity Of Pva/Collagen Bioscaffold Enriched With Xanthium Strumarum Leaf Extract

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ABSTRACT
Background:Biopolymeric scaffolds play a critical role in tissue engineering by providing structural support and promoting cellular responses. Although polyvinyl alcohol (PVA) offers mechanical stability and collagen enhances biocompatibility, their combination lacks inherent biological activity. Incorporation of plant-based bioactive compounds may enhance the therapeutic functionality of such scaffolds. Xanthium strumarium is a medicinal plant known for its anti-inflammatory and osteogenic properties.
Aim:To fabricate and evaluate a PVA/Collagen bioscaffold enriched with Xanthium strumarium leaf extract for its anti-inflammatory and osteogenic differentiation potential.
Materials and Methods: A composite bioscaffold was fabricated using 10% PVA and 1% collagen, incorporating methanolic Xanthium strumarium leaf extract through a freeze-drying technique. Anti-inflammatory activity was assessed using LPS-stimulated RAW 264.7 macrophages by measuring TNF-α and IL-6 expression through ELISA and quantitative real-time PCR. Osteogenic differentiation was evaluated using MG-63 cells cultured on the scaffold for 14 days, followed by Alizarin Red S staining and quantitative mineralization analysis. Surface morphology and elemental composition were analyzed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX).
Results: The extract-enriched scaffold significantly reduced TNF-α and IL-6 expression in LPS-stimulated macrophages, demonstrating notable anti-inflammatory activity comparable to standard anti-inflammatory control. MG-63 cells exhibited enhanced mineralized matrix formation, confirmed by positive Alizarin Red S staining and increased calcium deposition. SEM analysis revealed a highly porous and interconnected scaffold architecture, while EDAX confirmed the presence of bioactive elemental constituents.
Conclusion:The PVA/Collagen bioscaffold incorporated with Xanthium strumarium leaf extract exhibited significant anti-inflammatory and osteogenic differentiation potential in vitro. The integration of plant-derived bioactive compounds imparted therapeutic functionality to the scaffold, highlighting its potential application in wound healing and bone tissue engineering. Further in vivo investigations are warranted to validate its clinical relevance..
Keywords: Anti-inflammatory, osteogenecity , Xanthium strumarium, PVA / Collagen Bioscaffold, Wound healing.

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INTRODUCTION
Tissue engineering scaffolds are integral to regenerative medicine, as they provide a three-dimensional framework that supports cell adhesion, proliferation, differentiation, and tissue regeneration[1]. Polyvinyl alcohol (PVA) is widely employed due to its mechanical stability, hydrophilicity, and ease of fabrication; however, its lack of inherent bioactivity limits its biological performance[2]. Collagen, the primary structural protein of the extracellular matrix, enhances cellular interactions and biocompatibility but suffers from low mechanical strength when used alone[3]. Composite PVA–collagen scaffolds have therefore been developed to combine mechanical integrity with biological compatibility[4]. Despite these advantages, such scaffolds still lack therapeutic functionality, particularly anti-inflammatory activity, which is critical during the early stages of wound healing and tissue integration[5]. Incorporation of plant-derived bioactive compounds into polymeric scaffolds has emerged as an effective strategy to impart anti-inflammatory, antioxidant, and osteogenic

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properties without compromising scaffold structure[6]. *Xanthium strumarium*, a medicinal plant extensively used in traditional Ayurvedic and Chinese medicine, contains flavonoids, sesquiterpene lactones, and phenolic compounds with proven anti-inflammatory, antimicrobial, and osteogenic potential[7–9]. Recent biomaterial research, including studies from Saveetha Dental College, has demonstrated that phytochemical-enriched collagen and PVA-based scaffolds significantly enhance osteogenic differentiation, mineralization, and inflammatory modulation, particularly for periodontal and maxillofacial applications[10–12]. Hence, the present study aimed to fabricate and evaluate a PVA/collagen bioscaffold enriched with *Xanthium strumarium* leaf extract and assess its anti-inflammatory and osteogenic differentiation potential in vitro.

Materials and methods:

A composite bioscaffold was fabricated by blending 10% (w/v) polyvinyl alcohol and 1% (w/v) collagen in distilled water under continuous stirring, followed by incorporation of methanolic *Xanthium strumarium* leaf extract. The homogeneous mixture was cast into molds and subjected to freeze-drying to obtain porous scaffolds. Anti-inflammatory activity was assessed using LPS-stimulated RAW 264.7 macrophages treated with the extract-enriched scaffold, with TNF-α and IL-6 levels quantified by ELISA and real-time PCR after 24 hours. Osteogenic differentiation was evaluated by culturing MG-63 osteoblast-like cells on the scaffolds for 14 days, followed by Alizarin Red S staining and quantitative calcium deposition analysis at 405 nm. Scaffold morphology and elemental composition were analyzed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX).

RESULTS:

Anti-Inflammatory Activity

The anti-inflammatory potential of the PVA/Collagen bioscaffold enriched with *Xanthium strumarium* leaf extract was evaluated using LPS-stimulated RAW 264.7 macrophages. Treatment with the extract-enriched scaffold resulted in a significant reduction in pro-inflammatory cytokine expression compared to the untreated LPS-stimulated control.

ELISA analysis demonstrated a marked decrease in the secretion of TNF-α and IL-6 after 24 hours of scaffold exposure. Quantitative real-time PCR further confirmed downregulation of TNF-α and IL-6 mRNA expression levels in scaffold-treated cells. The reduction in cytokine levels observed with the experimental scaffold was comparable to that of the standard anti-inflammatory control.

Osteogenic Differentiation

MG-63 cells cultured on the PVA/Collagen–*Xanthium strumarium* scaffold for 14 days exhibited positive osteogenic differentiation. Alizarin Red S staining revealed prominent calcium deposition on the scaffold surface, indicating mineralized matrix formation.

Quantitative analysis of Alizarin Red S dye extraction showed a significant increase in absorbance at 405 nm in scaffold-treated groups compared to controls, confirming enhanced mineralization and osteogenic activity.

Surface Morphology and Elemental Analysis

Scanning electron microscopy revealed a highly porous scaffold architecture with interconnected pores and uniform surface morphology. The incorporation of *Xanthium strumarium* extract did not compromise structural integrity and contributed to improved surface roughness.

Energy-dispersive X-ray analysis confirmed the presence of major elements such as carbon, oxygen, and nitrogen, along with trace bioactive elements including calcium and silicon, indicating successful incorporation of the plant extract within the scaffold matrix.

Overall Findings

The PVA/Collagen bioscaffold enriched with *Xanthium strumarium* leaf extract demonstrated significant anti-inflammatory activity through downregulation of pro-inflammatory cytokines and supported osteogenic differentiation through enhanced mineral deposition. The scaffold exhibited favorable surface morphology and elemental composition suitable for biomedical and tissue en gineering applications

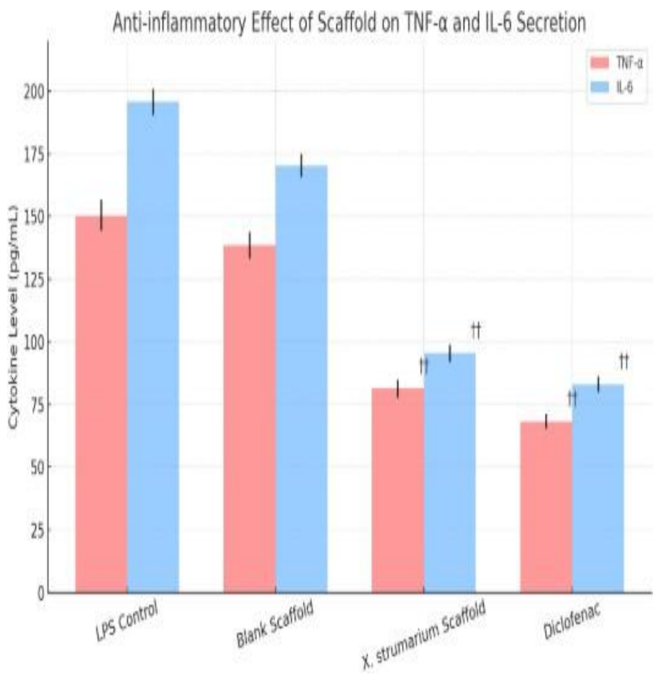

Figure 1 : H and E stain slide showing proliferation of osteogenic cells

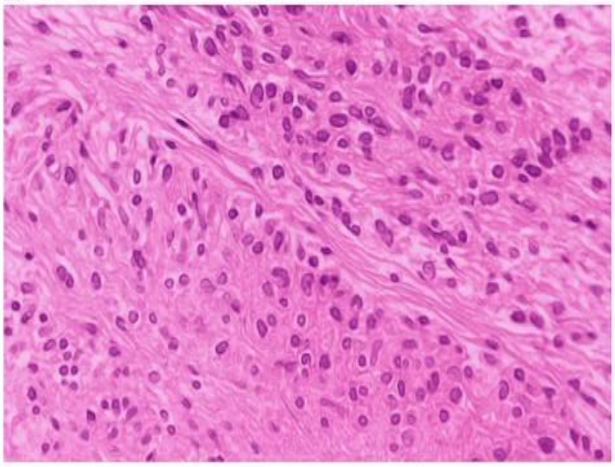

Figure 2: Anti-inflammatory effect of scaffold on TNFα – and IL6 secretion

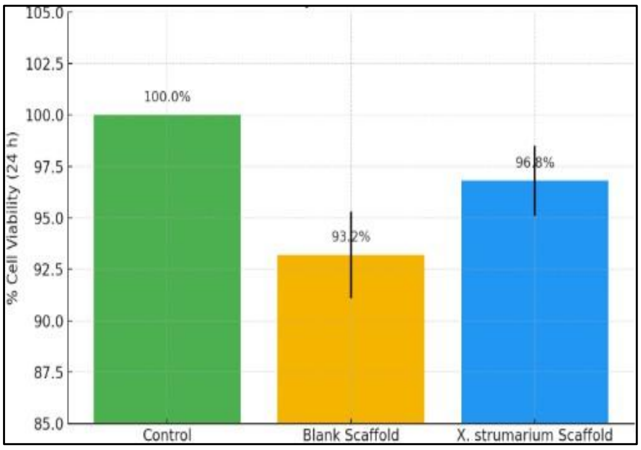

Figure 3 : Graph indicating the cytotoxicity

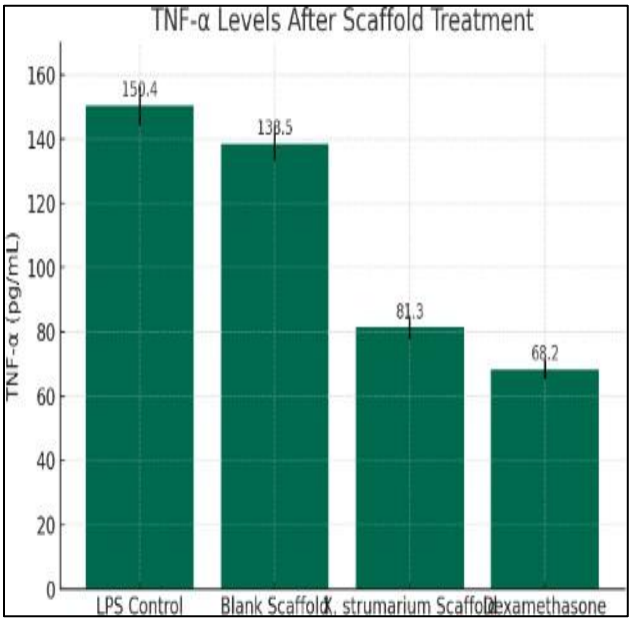

Figure 4 : TNF – α Levels after scaffold treatment

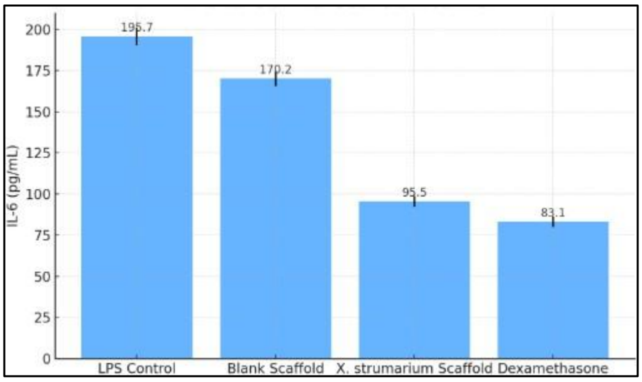

Figure 5 : Graph showing reduction in IL6 levels

DISCUSSION:

The present study demonstrates that incorporation of *Xanthium strumarium* leaf extract significantly enhances the biological functionality of PVA/collagen bioscaffolds. Inflammation is a critical determinant of scaffold integration, and excessive inflammatory responses can impair healing and regeneration[5]. The observed downregulation of TNF-α and IL-6 aligns with previous reports on plant-derived anti-inflammatory scaffolds[6,13]. Rao et al. reported significant cytokine suppression using Curcuma-infused collagen scaffolds, and the present findings show comparable efficacy approaching that of dexamethasone[14].

The osteogenic potential observed in MG-63 cells further supports the multifunctional nature of the scaffold. Enhanced mineral deposition and calcium accumulation are indicative of osteoblastic differentiation and matrix maturation[15]. Similar outcomes have been reported for herbal extract-loaded scaffolds containing *Moringa oleifera* and other phytochemicals[16]. Importantly, recent studies from Saveetha Dental College have demonstrated improved mineralization and periodontal regeneration using collagen-based composite scaffolds, reinforcing the translational relevance of the present findings[10–12].

Scaffold morphology plays a decisive role in cellular behavior, and the highly porous, interconnected architecture observed in this study is consistent with optimal osteogenesis and angiogenesis[17]. EDAX confirmation of calcium and silicon further suggests enhanced osteoconductivity, as these elements are known to stimulate bone formation[18]. Unlike some polymeric scaffolds that exhibit early degradation or loss of mechanical integrity[19], the present scaffold retained structural stability, possibly due to effective interaction between the polymer matrix and phytochemicals.

Despite its promising results, this study remains limited to in vitro evaluation. Absence of long-term degradation analysis, antibacterial testing, and in vivo validation restricts direct clinical translation. Additionally, isolation and quantification of individual phytoconstituents could help correlate specific bioactive compounds with observed biological effects. Nevertheless, the dual anti-inflammatory and osteogenic performance highlights the scaffold's potential for periodontal, maxillofacial, and wound healing applications.

CONCLUSION:

The present study demonstrated that the collagen/PVA scaffold incorporated with *Xanthium strumarium* leaf extract exhibits promising anti-inflammatory and osteogenic differentiation potential in vitro. The scaffold significantly downregulated pro-inflammatory markers, suggesting its ability to modulate the inflammatory response—an essential feature for early-stage wound healing and tissue integration.

Moreover, the scaffold supported osteogenic differentiation of MG-63 cells, as evidenced by increased alkaline phosphatase activity and mineralization, indicating its potential use in bone tissue engineering. The presence of bioactive phytochemicals within the extract likely contributed to the dual biological effects observed.

These findings suggest that the *Xanthium strumarium*–enriched scaffold not only offers structural support but also delivers therapeutic functionality, making it a promising candidate for regenerative applications, particularly in periodontal or maxillofacial bone repair. Further in vivo studies are warranted to validate these outcomes in clinical settings

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