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COMPARATIVE MODULATION OF SYSTEMIC INFLAMMATORY AND OXIDATIVE STRESS BIOMARKERS BY GREEN-SYNTHESIZED SIO_2 NANOPARTICLES AND RHUS CORIARIA IN A SHEEP TIBIAL DEFECT MODEL

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ABSTRACT

Background: Bone regeneration is strongly influenced by systemic inflammatory mediators and oxidative stress status. Research has discovered plant extracts from nature and nanoparticles produced through green synthesis which demonstrate ability to manage biochemical reactions that take place during bone healing processes. **Objective:** This study aimed to evaluate how sumac extract (Rhus coriaria L.) and bio-synthesized silicon dioxide nanoparticles (SiO_2 NPs) influence serum inflammatory and oxidative stress markers which were measured in surgically induced tibial bone defects in sheep. **Methods:** Twenty-eight clinically healthy male sheep were randomly allocated without health issues into three groups which included control animals and two treatment groups receiving sumac and bio-synthesized SiO_2 NP respectively. Each group was further evaluated at immediate postoperative reference point (Time-0), 1 month, and 2 months post-operation (n = 4 per time point). The researchers measured IL-6 and TNF-α levels in serum to assess inflammatory biomarkers and they used MDA and SOD and T-AOC to assess oxidative stress and antioxidant defense. **Results:** Untreated animals exhibited the highest inflammatory cytokines and oxidative imbalance. The two compounds sumac extract and SiO_2 nanoparticles showed time-dependent decreases in IL-6 and TNF-α and MDA while they simultaneously increased SOD activity and T-AOC. The SiO_2 NP group showed the most significant biochemical improvements which became evident during the 2-month period. **Conclusion:** The research data showed that green bio-synthesized SiO_2 nanoparticles was associated with greater improvement for decreasing inflammatory markers and oxidative stress indicators when compared to using sumac extract as a single. The research findings show that these materials have shown promise as additional bone healing materials which can improve chemical conditions during bone recovery but need more extensive research to confirm their effectiveness.

KEYWORDS: Bone regeneration; Rhus coriaria; Bio-synthesized SiO_2 nanoparticles; Inflammatory cytokines; Oxidative stress; Antioxidant defense; Tibial bone defect; Sheep model.

INTRODUCTION

Bone regeneration involves more than physical repair because it follows a biological schedule which depends on the interaction between inflammatory substances and the equilibrium between oxidative stress. Surgical bone injury triggers a wide-ranging systemic reaction which produces detectable cytokines and redox biomarkers that exist in blood circulation to help track healing development or deterioration. The scientific community depends on serum-based inflammatory and antioxidant indicators which demonstrate high reliability for evaluating bone repair quality through research conducted on large animal orthopedic models (Maruyama et al., 2020).

The initial biological process of bone healing starts with inflammation but this process becomes harmful when it continues beyond its initial stage. Pro-inflammatory cytokines such as IL-6 and TNF-α play dual roles which include interleukin-6 (IL-6) and tumor necrosis factor-

alpha (TNF-α) that function in two ways. The body uses IL-6 and TNF-α to begin immune cell migration and waste elimination but their prolonged presence causes delayed bone development and matrix deterioration (Khosla and Monroe, 2018). Results indicates that high TNF-α concentrations stop osteoblast cells from developing but they activate osteoclast cells to perform bone destruction instead of bone repair (Yin et al., 2025). The body repairs bones through two separate mechanisms which include oxidative stress as a vital biochemical process. The body produces excessive reactive oxygen species (ROS) during surgical trauma and inflammatory responses which exceed the capacity of its natural antioxidant system to neutralize. The compound Malondialdehyde (MDA) serves as a marker for oxidative damage because it forms during lipid peroxidation and affects collagen production and bone mineral density (Woźniak et al., 2025). Antioxidant enzymes such as SOD and total antioxidant capacity (T-AOC) counteract ROS accumulation superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) which support osteogenic differentiation in the microenvironment (Soe et al., 2025).

Biomaterials demonstrates that nanostructured compounds which include silicon dioxide nanoparticles (SiO_2 NPs) show regenerative abilities because they influence immune system reactions and protect cells from damage. The process of green synthesis using plant extracts allows scientists to develop nanoparticles which show better biocompatibility because they produce lower inflammation and stronger antioxidant protection (Singaravelu et al., 2025). Rhus coriaria (sumac) contains polyphenol compounds which show promise for bone treatment supplements because they have anti-inflammatory effects and free radical scavenging capabilities (Alsamri et al., 2021).

The evaluation of serum inflammatory cytokines (IL-6, TNF-α) together with oxidative stress biomarkers (MDA, SOD, T-AOC) helps scientists to determine how natural extracts applied topically and bio-synthesized nanomaterials affect the entire healing process. The study monitored serum biochemical marker changes in sheep with tibial defects who received sumac extract or green-synthesized SiO_2 nanoparticles to evaluate their effects on bone healing through their ability to modify systemic inflammatory-oxidative responses.

MATERIALS AND METHODS

Study Design and Experimental Framework

The study used controlled experimental methods to study how local biomaterial affects systemic inflammation and oxidative stress in sheep with surgically created tibial bone defects. The design followed a healing process timeline which studied biological changes at different time points after surgery instead of using a single measurement point. The study used serum biomarkers which measured inflammation and redox balance to study how the body heals at a systemic level.

The study used random assignment to place animals into experimental groups which minimized selection bias and established equal numbers of animals across all groups. Two postoperative time points, one month and two months, were adopted in order to capture both the early inflammatory phase and the subsequent remodeling stage of bone regeneration.

Experimental Animals and Housing Conditions

Clinically healthy male sheep were selected for this study because their size and bone regeneration abilities made them appropriate for investigating orthopedic and regenerative medical treatments. The study used animals which shared similar ages and weights to minimize biological differences between subjects.

The sheep needed to adapt to their environment through a controlled acclimatization process before surgeons performed their surgeries. The study facilities operated with controlled environmental conditions which included fixed temperature settings and ventilation systems and light-dark cycle patterns. The animals received commercial balanced diet and clean water access throughout the entire study period. The team performed continuous veterinary examinations to confirm that no major illness or stress-induced health issues affected the animals. Future research needs to perform complete physicochemical testing of SiO_2 nanoparticles which will establish biomaterial reproducibility and follow international nanomedicine reporting standards. The study needs to perform complete physicochemical testing of synthesized SiO_2 nanoparticles through DLS for size distribution and TEM/SEM for morphology and zeta potential measurement for surface charge and FTIR and XRD for structural verification. The current pilot study needs additional parameters which future confirmatory must include.

Experimental Grouping

A total of 28 Clinically healthy male sheep (aged 10–12 months, weighing approximately 25–30 kg) used as the experimental model were randomly allocated into main experimental groups. The groups were further subdivided according to the postoperative evaluation time points (Time-0, 1 month, and 2 months), with four animals assigned to each time point (n = 4/time point). Control animals were also subdivided into identical postoperative evaluation periods (Time-0, 1 month, and 2 months; n=4 each).

The researchers established Time-0 as the moment when they collected post-defect reference samples right after surgical induction and local material application instead of using it to measure pre-operative physiological conditions. Fixed doses of sumac extract and SiO_2 nanoparticles were applied per defect throughout all animals. “The researchers applied 0.2 mL of sumac extract at a concentration of 10 mg/mL to each defect for a total dose of 2 mg per defect. The nanoparticle group received 5 mg of green-synthesized SiO_2 nanoparticles

per defect which they applied directly to the defect site using an appropriate delivery method to maintain the particles inside the defect. These parameters were explicitly reported to enhance methodological reproducibility and facilitate inter-study comparison.

The present investigation was designed as a preliminary biological evaluation study; therefore, advanced physicochemical characterization analyses were reserved for subsequent mechanistic investigations.

Negative Control Group (Untreated Tibial Bone Defect; n = 4 per evaluation period
Control animals were evaluated at the same postoperative intervals adopted for the treated groups in order to ensure temporal comparability of systemic biochemical responses.

1. **Sumac Extract Group (Rhus coriaria L.; n = 12)**

The tibial defect was locally treated with sumac extract. Animals were euthanized and sampled at.

- **Time-0 (immediate postoperative reference point, immediately after defect creation; n = 4)**
- **1-month post-operation (n = 4)**
- **2 months post-operation (n = 4)**

2. **Bio-Synthesized SiO_2 Nanoparticles Group (Green-Synthesized SiO_2 NPs using Rhus coriaria extract; n = 12)**

Defects were locally treated with bio-synthesized silicon dioxide nanoparticles. Animals were evaluated at.

- **Time-0 (immediate postoperative reference point, immediately after defect creation; n = 4)**
- **1-month post-operation (n = 4)**
- **2 months post-operation (n = 4)**

This method used to study how bone repair biomarkers changed over time between defects that received no treatment and those that received local treatment. This method allowed the researchers to study both initial and subsequent biochemical responses which occurred during bone healing. The researchers established Time-0 as the moment when they collected post-defect reference samples right after surgical induction and local material application instead of using it to measure pre-operative physiological conditions. The biochemical results from this period show how the body reacts to surgical trauma during its initial response to medical instead of its typical pre-operative values. The team needs to handle statistical results from this small study with careful attention because the results need to be viewed with reserve. The study presents exploratory separation results through superscript lettering because the findings do not establish definitive conclusions.

Surgical Procedure and Defect Induction
The surgical team conducted all procedures in sterile environments while using their established orthopedic instruments. The animals received ketamine (10 mg/kg) and xylazine (2 mg/kg) through intramuscular injection for anesthesia while lidocaine with epinephrine was used for local infiltration to achieve better pain management and reduce surgical bleeding.

The first step of the procedure involved making a longitudinal cut in the skin which led to the tibial area before the bone became accessible through careful bone dissection. The periosteum received proper elevation to maintain its ability to generate new bone tissue. The surgical bur operated under sterile saline irrigation made an 8 mm diameter and 5 mm deep standardized critical-sized tibial defect.

The material either sumac extract or bio-synthesized SiO_2 nanoparticles received local application after the defect preparation process. The periosteum received its new position before the surgeon completed the wound closure through multiple non-absorbable suture layers.

Postoperative Management
The animals received standard postoperative treatment which included antibiotic spray application to their wounds and therapeutic antibiotic prophylaxis through systemic administration. The surgical areas received daily inspections to check for any signs of infection or inflammation or wound dehiscence. The surgical team removed the sutures after seven days when the tissue had achieved its correct closure stage.

Blood Sampling and Serum Preparation
The experimental team collected venous blood samples through sterile procedures at the pre-determined experimental time points. Blood was allowed to clot at room temperature and subsequently centrifuged to obtain serum. The serum samples received storage at −20°C temperatures until scientists performed their biochemical tests.

Assessment of Serum Inflammatory Biomarkers
Systemic inflammatory status was evaluated by serum tests to determine interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels for evaluating the extent of systemic inflammation. From China, Elabscience made ELISA kits according to the manufacturer's instructions, including calibration standards and duplicate sample analysis to minimize inter-assay variability. Following the guidance offered by the manufacturer, each step in the assay process was done exactly as suggested. A machine called the microplate ELISA reader took readings by measuring light signals in the plates. Testing oxidative stress and antioxidant defenses involved looking at levels of certain markers. MDA levels gave scientists a way to track how much damage oxidatives had done across lipids. That substance acts like a report card for overall lipid health, showing where harm has taken place. Looking at superoxide dismutase activity gave insight into how bodies handle oxidative stress. Total antioxidant capacity was also assessed using established methods. Each assay was repeated twice to ensure consistency across tests. Standard calibration

curves guided the measurement process. These steps supported reliable data collection during testing.

Biochemical Assay Kits and Equipment

Testing of biological samples relied on established commercial assay kits proven for accuracy. Equipment used in labs included centrifuges, UV–visible spectrophotometers, ELISA washers, microplate readers - all standard tools helping achieve reliable outcomes.

Statical analysis

Analysis involved comparing outcomes across different groups while accounting for potential variations. Instead of treating groups as identical at first, differences between them shaped how findings were interpreted. Exploratory statistical comparisons were performed using two-way ANOVA followed by Tukey's post-hoc test to evaluate temporal and treatment-related biochemical trends. Due to the pilot-scale sample size (n = 4 per subgroup), findings should be interpreted cautiously as preliminary biological observations rather than definitive statistical conclusions. Due to the pilot-scale nature of the study and limited subgroup size, statistical outputs should be interpreted as exploratory trend analyses rather than confirmatory inferential evidence.

Ethical Approval

All experimental procedures involving animals were conducted in accordance with internationally accepted ethical standards for the use of animals in biomedical. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC), College of Dentistry, University of Mosul, Iraq (Approval No. 145/2025). The researchers took all necessary steps to prevent animal distress while keeping the animals calm and using proper animal handling techniques from the start of surgery through the end of sample retrieval.

RESULTS

Inflammatory Biomarkers (IL-6 and TNF-α)

The treated groups showed a gradual reduction in their serum IL-6 levels after surgery when compared to the untreated defect animals. The IL-6 levels in the negative control group reached their highest point at 181.9 ± 43.5 ng/L but sumac-treated animals showed decreasing IL-6 levels which reached 90.0 ± 19.8 ng/L at the 2-month mark. The bio-synthesized SiO_2 nanoparticle group exhibited the lowest values in IL-6 levels which achieved its minimum value at 2 months (75.0 ± 15.0 ng/L). These findings indicate a reduction in systemic inflammatory activity through biomaterial applications to particular areas (Table 1, Figure 1).

A comparable trend was observed for TNF-α. The serum TNF-α concentration in untreated animals reached its highest point at 357.5 ± 35.0 ng/L. The Sumac extract treatment levels decreased at a moderate rate throughout the postoperative period until they reached 265.3 ± 20.9 ng/L at 2 months. The bio-synthesized SiO_2 nanoparticle group showed the most significant decrease in their TNF-α levels which reached 160.0 ± 18.0 ng/L during the 2-month assessment. Treated groups exhibited lower cytokine levels compared with controls throughout the entire healing process (Table 1, Figure 1).

Table 1: Serum Inflammatory Biomarkers Across Experimental Groups.

Experimental Group (Full Scientific Name)	IL-6 (ng/L) Mean ± SD	TNF-α (ng/L) Mean ± SD
Negative Control (subdivided temporally) Group (Untreated Defect)	181.9 ± 43.5^{a}	357.5 ± 35.0^{a}
Sumac Extract – immediate postoperative reference point (Time-0)	135.0 ± 20.8^{b}	345.0 ± 5.0^{ab}
Sumac Extract – 1 Month Post-Operation	112.5 ± 10.6^{bc}	323.0 ± 32.5^{b}
Sumac Extract – 2 Months Post-Operation	90.0 ± 19.8^{c}	265.3 ± 20.9^{c}
Bio-Synthesized SiO_2 NPs – immediate postoperative reference point (Time-0)	109.5 ± 10.6^{bc}	250.0 ± 21.2^{c}
Bio-Synthesized SiO_2 NPs – 1 Month Post-Operation	98.0 ± 42.4^{c}	220.0 ± 35.4^{d}
Bio-Synthesized SiO_2 NPs – 2 Months Post-Operation	75.0 ± 15.0^{cd}	160.0 ± 18.0^{e}

Values are expressed as mean ± SD (n = 4). Different superscript letters indicate exploratory statistical separation between groups at $p \leq 0.05$.

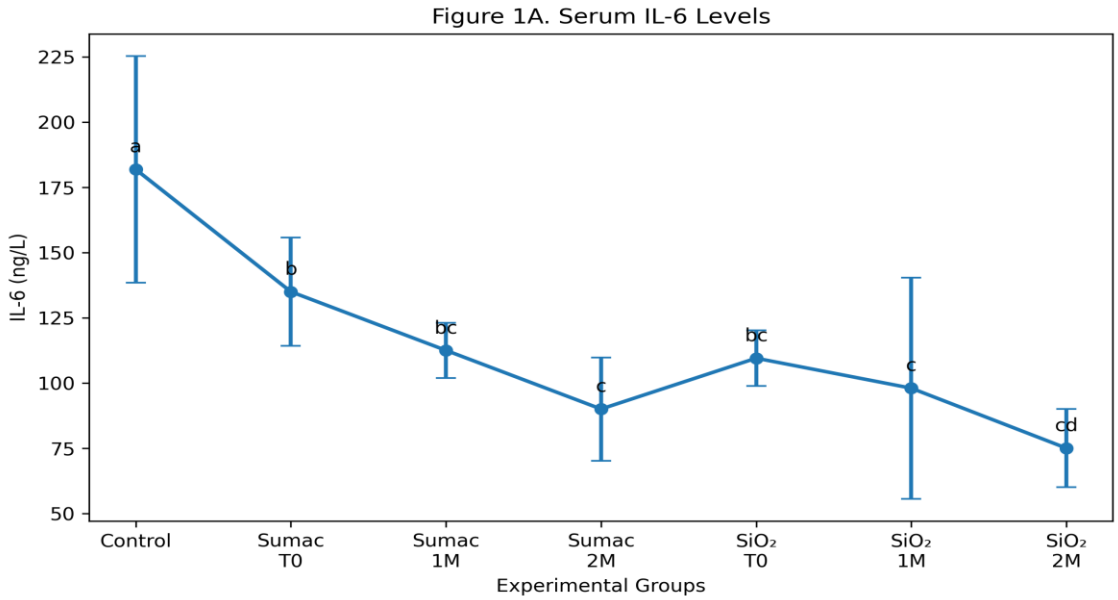

Figure 1A: Time-Dependent Changes in Serum Inflammatory Cytokines (IL-6) in Treated and Untreated Sheep Tibial Bone Defects.

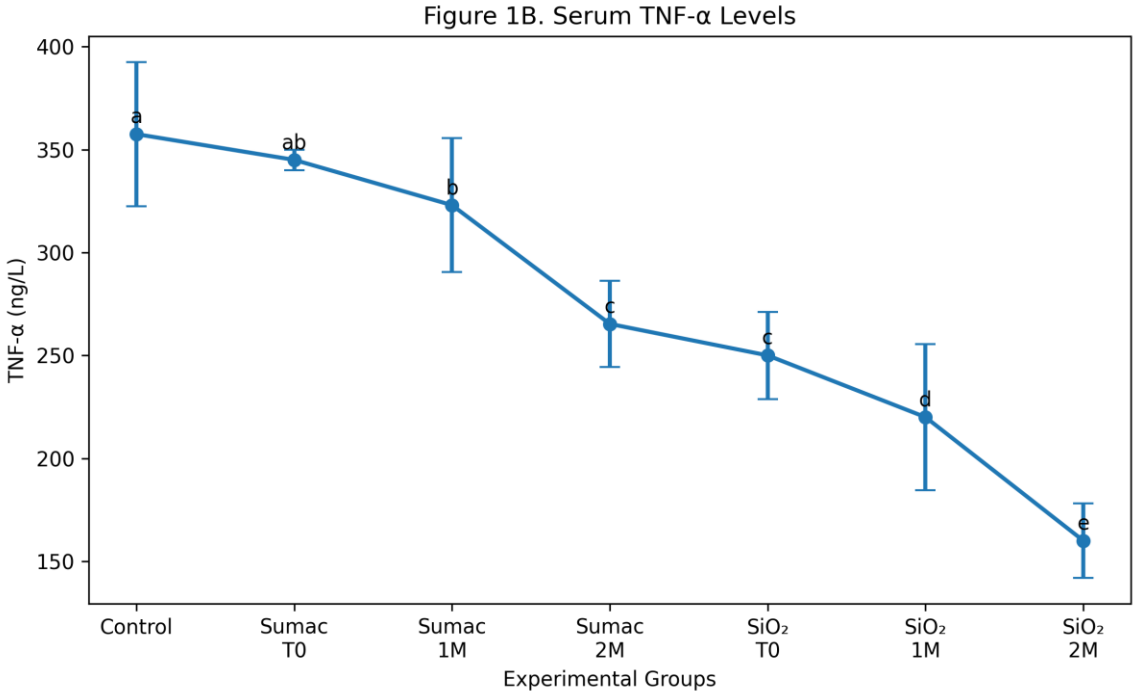

Figure 1B: Time-Dependent Changes in Serum Inflammatory Cytokines (TNF-α) in Treated and Untreated Sheep Tibial Bone Defects.

Oxidative Stress and Antioxidant Defense Markers

The untreated control group showed the highest serum malondialdehyde (MDA) levels which reached 238.8 ± 48.2 ng/mL because their lipid peroxidation levels increased after defect creation without any treatment. The MDA values in Sumac-treated animals showed a steady decline from the start of the study until they reached their lowest point at 2 months (140.0 ± 28.3 ng/mL). The bio-synthesized SiO_2 nanoparticle group showed the lowest postoperative MDA concentrations which measured 97.0 ± 20.0 ng/mL at 2 months (Table 2, Figure 2A).

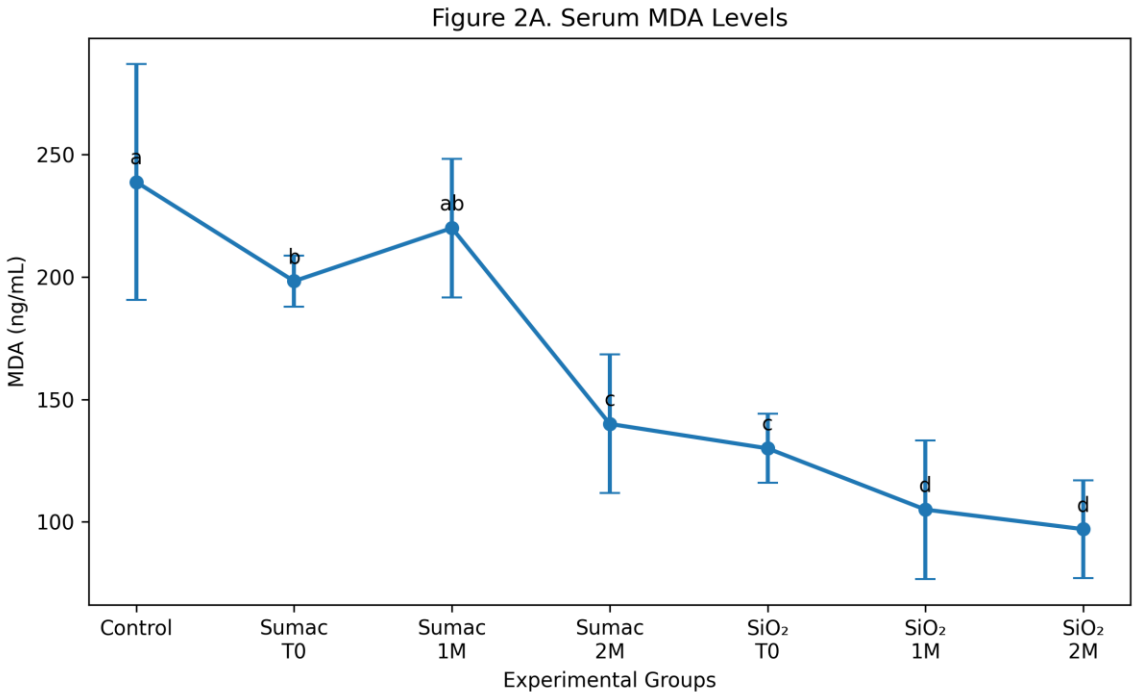

Figure 2A: Serum Malondialdehyde (MDA) Levels Across Experimental Groups Following Local Application of Sumac Extract and Green-Synthesized SiO_2 Nanoparticles in a Sheep Tibial Defect Model.

Antioxidant defense markers showed an opposite pattern to oxidative stress indices. The control group displayed the lowest SOD activity which measured at 1035 ± 458 pg/mL and the group demonstrated the lowest total antioxidant capacity at 0.576 ± 0.058 µmol/mL. The therapeutic methods resulted in higher antioxidant levels which reached their peak during the last assessment period. The bio-synthesized SiO_2 nanoparticle group showed the highest improvement at 2 months when SOD levels reached 2925 ± 120 pg/mL and T-AOC levels reached 1.971 ± 0.050 µmol/mL (Table 2, Figure 2 B).

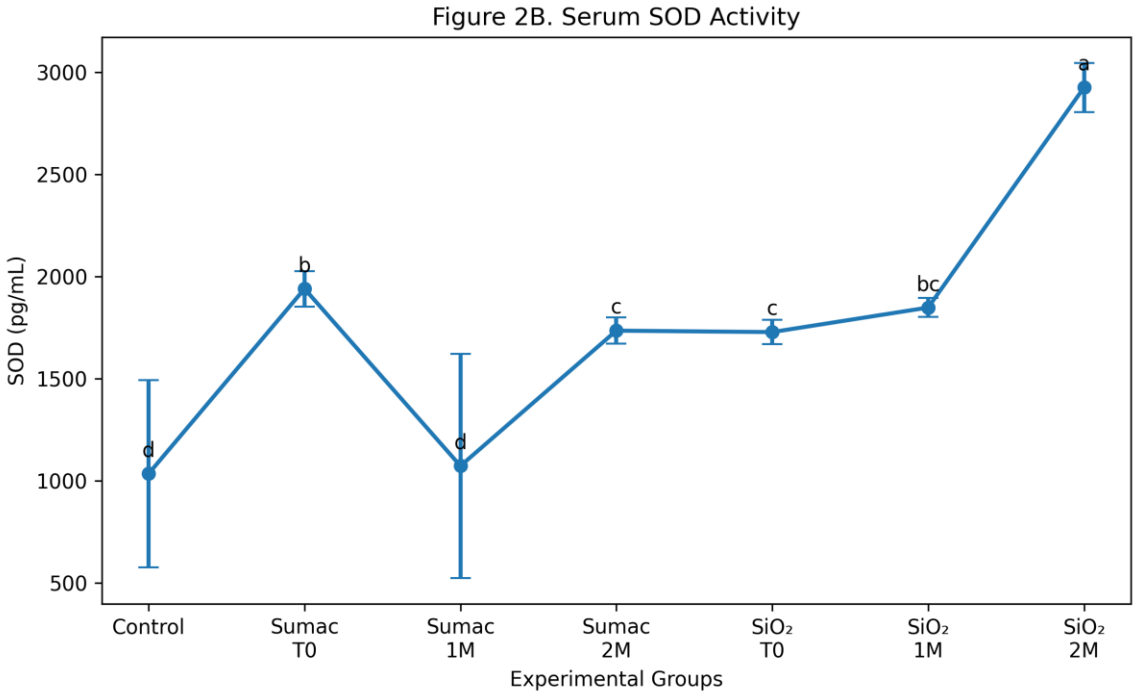

Figure 2B: Serum Superoxide Dismutase (SOD) Activity Across Experimental Groups Following Local Application of Sumac Extract and Green-Synthesized SiO_2 Nanoparticles in a Sheep Tibial Defect Model.

The results received support from total antioxidant capacity (T-AOC) measurement results. The antioxidant reserve of untreated animals reached its lowest point at 0.576 ± 0.058 µmol/mL but sumac led to a substantial increase in T-AOC which reached its highest point at 2 months (1.498 ± 0.027 µmol/mL). The bio-synthesized SiO_2 nanoparticles achieved the highest enhancement which produced the maximum antioxidant capacity at 2 months (1.971 ± 0.050 µmol/mL). The statistically confirmed elevation shows that nanoparticle treatment creates an improved chemistry which supports bone healing through its ability to enhance antioxidant defense systems (Table 2).

The statistical evaluation showed that serum MDA concentration measurements between groups demonstrated substantial differences in their oxidative stress levels ($p \leq 0.05$). The post-hoc tests conducted by Tukey revealed that both treatment modalities led to decreased lipid peroxidation levels when compared to the control group which received no treatment. The bio-synthesized SiO_2 nanoparticle produced the lowest MDA values which were measured in animals at 2 months.

The antioxidant defense markers showed a different significant pattern which ran contrary to the other results. The treated groups demonstrated higher SOD activity and total antioxidant capacity (T-AOC) which exceeded control group values at a statistical significance of $p \leq 0.05$ (Table 2). The experimental results led to improved redox system stability which helped the bone healing process.

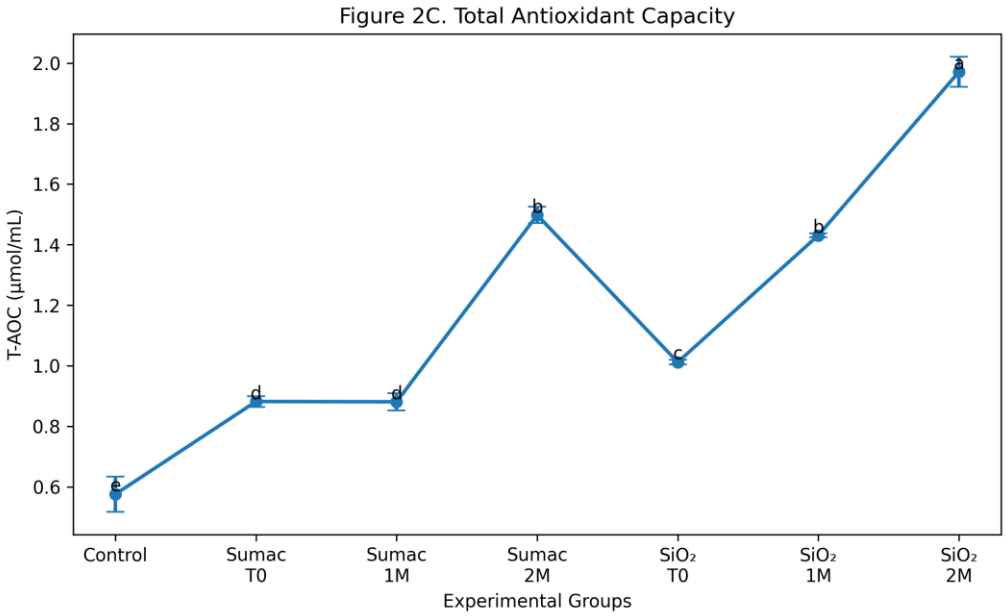

Figure 2 C: Total Antioxidant Capacity (T-AOC) Across Experimental Groups Following Local Application of Sumac Extract and Green-Synthesized SiO_2 Nanoparticles in a Sheep Tibial Defect Model.

Table 2: Serum Oxidative Stress and Antioxidant Defense Markers.

Experimental Group (Full Scientific Name)	MDA (ng/mL) Mean ± SD	SOD (pg/mL) Mean ± SD	T-AOC (µmol/mL) Mean ± SD
Negative Control (subdivided temporally) Group (Untreated Defect)	238.8 ± 48.2^{a}	1035 ± 458^{d}	0.576 ± 0.058^{e}
Sumac Extract – immediate postoperative reference point (Time-0)	198.3 ± 10.4^{b}	1940 ± 87^{b}	0.882 ± 0.018^{d}
Sumac Extract – 1 Month Post-Operation	220.0 ± 28.3^{ab}	1073 ± 548^{d}	0.881 ± 0.028^{d}
Sumac Extract – 2 Months Post-Operation	140.0 ± 28.3^{c}	1735 ± 64^{c}	1.498 ± 0.027^{b}
Bio-Synthesized SiO_2 NPs – immediate postoperative reference point (Time-0)	130.0 ± 14.1^{c}	1728 ± 60^{c}	1.012 ± 0.008^{c}
Bio-Synthesized SiO_2 NPs – 1 Month Post-Operation	105.0 ± 28.3^{d}	1848 ± 46^{bc}	1.431 ± 0.007^{b}
Bio-Synthesized SiO_2 NPs – 2 Months Post-Operation	97.0 ± 20.0^{d}	2925 ± 120^{a}	1.971 ± 0.050^{a}

Values are expressed as mean ± SD (n = 4). Different superscript letters within the same column indicate exploratory statistical differences between groups at $p \leq 0.05$.

DISCUSSION

Bone regeneration is regulated not only by local structural remodeling, but also by systemic inflammatory and oxidative mechanisms that influence tissue repair quality. The present findings suggest that both sumac extract and green-synthesized SiO_2 nanoparticles were associated with modulation of circulating inflammatory cytokines and antioxidant defense biomarkers during postoperative bone healing.

Inflammatory Modulation and Cytokine Suppression

The inflammatory response starts bone healing but extended or increased cytokine levels interfere with osteogenic development which results in longer healing times for bone defects. The findings demonstrated that untreated animals developed the most severe serum IL-6 and TNF-α levels because their bodies maintained uncontrolled systemic inflammation due to bone damage. The findings confirm earlier studies which demonstrated that TNF-α activation beyond a certain point prevents osteoblast cell development but leads to osteoclast cell growth which results in bone deterioration instead of bone construction (Xu et al., 2023). The IL-6 and TNF-α levels in sumac-treated groups decreased steadily from the start of the study until they reached their lowest point at two months after surgery. Rhus coriaria fights inflammation by using its polyphenol compounds which decrease cytokine levels because these compounds have proven their ability to block NF-κB triggered inflammation and lower cytokine production (Chojnacka and Lewandowska, 2023).

The mechanistic approach needs to control inflammatory persistence during later remodeling stages because IL-6 overexpression results in weak collagen synthesis and prolonged mineralization process (Jiang et al., 2024). The bio-synthesized SiO_2 nanoparticle groups showed the most effective IL-6 and TNF-α suppression which reached its lowest point during the two-month period. The nano-based interventions will produce better immunomodulatory results because they support osteoimmune equilibrium and protect against oxidative stress which cytokines trigger. Studies demonstrate that silicon-based nanostructures in biomaterials reduce inflammatory mediator production while they improve osteogenic cell differentiation (Kong et al., 2022).

Oxidative Stress Reduction and Antioxidant Reinforcement

Bone regeneration faces growing evidence that oxidative stress functions as a major blocking mechanism which affects bone healing processes especially when inflammation leads to increased reactive oxygen species (ROS) that damage cellular repair systems. The untreated control group displayed the highest MDA levels which demonstrated that tibial defects which receive no treatment result in the most severe systemic lipid peroxidation and oxidative stress. The study demonstrates that osteoblast cells experience death and their extracellular matrix structure becomes damaged when ROS levels reach their highest points (Shinohara et al., 2025). The two modalities reduced MDA levels but SiO_2 nanoparticle resulted in the greatest decrease which protected membranes from oxidative damage. The antioxidative effects of these nanoparticles occur because they remove ROS through indirect methods while they create stable cellular environments which support bone cell development (Suresh et al., 2024). The SOD activity measurements together with total antioxidant capacity (T-AOC) results show that these treatments protect cells from oxidative stress while enhancing the natural antioxidant defense mechanisms.

The SOD and T-AOC levels in the bio-synthesized SiO_2 group reached their peak during the entire two-month period. This observation may be biologically relevant because antioxidant enzymes contribute to osteogenic signaling pathways associated with RUNX2 activation and collagen matrix formation (Domazetovic et al., 2017). The observed antioxidant enhancement in this study appears to be a fundamental process which nano-interventions use to speed up tissue repair.

Comparative Interpretation: Sumac Versus Nano-Based Therapy

The biomarker normalization results showed better results when using bio-synthesized SiO_2 nanoparticles compared to sumac extract as a standalone treatment. The combination of plant-derived bioactive compounds with nanoscale physical and chemical properties produces enhanced systemic healing effects which phytotherapy cannot reach on its own. Previous studies have demonstrated that silicon-based biomaterials promote osteoblast cell growth while they help bones develop better mineral content and structural organization through environmentally friendly biocompatible manufacturing methods (Qi et al., 2019). The present serum test results show that nano-regenerative therapy applied at the local site creates two essential systemic effects which reduce both inflammatory stress and oxidative imbalance that impact bone remodeling results.

Study Limitations and Future Directions

Multiple biochemical trends became evident through observation yet need to recognize specific constraints in their study. The study results could be influenced by the limited number of animals per subgroup because this leads to decreased statistical power and reduced ability to detect small biomarker changes. Future studies need to confirm these results through studies which combine blood test results with direct bone regeneration evaluations that should measure bone structure healing through histomorphometry and micro-CT bone volume analysis and osteogenic marker assessments of alkaline phosphatase and osteocalcin. The research fails to include a sham-operated group which creates a major problem because surgical trauma might lead to brief increases in cytokine levels and oxidative stress activation. Including a sham comparator in future studies

would allow clearer discrimination between biomaterial-driven modulation and the physiological inflammatory response induced by defect creation itself.

Furthermore, detailed physicochemical characterization of the synthesized nanoparticles (e.g., particle size distribution, zeta potential, and structural confirmation) was not included within this report. Therefore, the findings should be interpreted as exploratory and hypothesis-generating, warranting confirmation through larger, mechanistically integrated studies.

CONCLUSION

The current pilot-scale findings suggest that green-synthesized SiO_2 nanoparticles, and to a lesser extent sumac extract, are associated with improved systemic inflammatory and oxidative biomarker profiles during the early stages of tibial defect healing in sheep. Nevertheless, larger-scale studies incorporating nanoparticle characterization, sham comparators, and direct bone regeneration endpoints are required before definitive translational conclusions can be drawn.

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Conflict of Interest: None.

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