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Original Article

Therapeutic Effects of Combined Zinc and α-Tocopherol Administration in a Rat Model of Staphylococcus aureus-Induced Sepsis

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ABSTRACT
Sepsis induces systemic inflammation through excessive production of proinflammatory cytokines, leading to oxidative stress, tissue damage, and multiorgan dysfunction. This study aimed to evaluate the synergistic effects of combined zinc and vitamin E (α-tocopherol) supplementation on inflammatory and biochemical parameters in *Staphylococcus aureus*-induced sepsis in male Wistar rats. Thirty rats were divided into six groups: (1) normal control, (2) Placebo control (sepsis without therapy), (3) positive control (levofloxacin 45 mg/kg BW + zinc 0.9 mg/kg BW + vitamin E 250 mg/kg BW), and (4–6) treatment groups receiving combined zinc (0.9, 1.8, and 2.7 mg/kg BW) with vitamin E (250 mg/kg BW). Sepsis was induced intraperitoneally, followed by treatment according to group. On day 9, serum levels of TNF-α, IL-6, CRP, AST, ALT, urea, creatinine, and albumin were analyzed, while lung and kidney, were examined histologically. The combination of zinc and vitamin E significantly decreased TNF-α, IL-6, and CRP levels while improving biochemical parameters and increasing serum albumin compared to the untreated group ($p \leq 0.05$). The highest efficacy was observed with zinc 2.7 mg/kg BW and vitamin E 250 mg/kg BW, which showed over 50% reduction in tissue damage, reduced inflammatory cell infiltration and interstitial hemorrhage in lung tissue, and improved hepatic cellular regeneration. These findings suggest that zinc and vitamin E exert synergistic anti-inflammatory and antioxidative effects, indicating their potential as adjuvant therapy in sepsis management.
Keywords: zinc, vitamin E, TNF-α, IL-6, CRP, sepsis, *Staphylococcus aureus*, oxidative stress, histopathology.

INTRODUCTION

Sepsis is a complex clinical syndrome characterized by a dysregulated host response to infection, leading to systemic inflammation, oxidative stress, and multiorgan dysfunction. Despite significant advances in critical care, sepsis remains one of the leading causes of mortality worldwide, accounting for nearly 11 million deaths annually approximately 20% of all global deaths[1]. The pathophysiology of sepsis involves an imbalance between proinflammatory and anti-inflammatory mediators, resulting in excessive cytokine release, endothelial dysfunction, mitochondrial damage, and organ failure[2]. Among the various pathogens that cause sepsis, *Staphylococcus aureus* is one of the most common and virulent bacteria, capable of inducing severe systemic infections due to its production of multiple toxins and immune-evasive mechanisms[3]. Experimental sepsis models using *S. aureus* have therefore become a reliable approach for evaluating potential therapeutic strategies.

The exaggerated inflammatory response in sepsis is primarily mediated by the release of cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP). These mediators promote leukocyte activation, increase vascular permeability, and initiate oxidative stress through the overproduction of reactive oxygen species (ROS)[4]. Persistent inflammation and oxidative damage contribute to hepatocellular injury, renal impairment, and pulmonary edema, which are hallmarks of multiorgan dysfunction in septic patients. Therefore, controlling cytokine production and reducing oxidative stress are critical strategies in the management of sepsis.

Nutritional immunomodulators such as zinc and vitamin E have attracted attention for their potential protective effects in sepsis due to their synergistic roles in antioxidant defense and immune regulation. Zinc is an essential trace element involved in over 300 enzymatic reactions, including antioxidant enzymes such as superoxide dismutase (SOD)[5]. Zinc deficiency has been associated with increased susceptibility to infection, elevated oxidative stress, and excessive inflammatory responses. Experimental studies have shown that zinc supplementation can suppress TNF-α and IL-6 production, stabilize cellular membranes, and enhance epithelial barrier integrity[6]. Furthermore, zinc modulates nuclear factor kappa-B (NF-κB) signaling, a key transcription factor responsible for cytokine gene expression during inflammation[7].

Vitamin E (α-tocopherol) is a lipid-soluble antioxidant that protects cellular membranes from lipid peroxidation and regulates immune responses. It acts as a scavenger of free radicals, thereby preventing oxidative injury to vital organs such as the liver, kidney, and lungs[8]. Vitamin E supplementation has been shown to attenuate inflammatory cytokine production and improve antioxidant enzyme activity in sepsis models[9]. Moreover, the combination of

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vitamin E with trace elements like zinc may offer synergistic protection, as zinc maintains intracellular antioxidant enzyme systems while vitamin E prevents lipid oxidation in membranes. Together, they may enhance the restoration of redox balance and suppress the cascade of inflammatory mediators.

Recent experimental evidence supports the hypothesis that the concurrent administration of zinc and vitamin E could modulate both oxidative and inflammatory pathways more effectively than either supplement alone. However, studies investigating this combination in S. aureus-induced sepsis remain limited, particularly in correlating biochemical, cytokine, and histopathological parameters. Therefore, the present study aimed to evaluate the therapeutic effects of combined zinc and α-tocopherol administration in male Wistar rats subjected to Staphylococcus aureus-induced sepsis. Specifically, this study assessed their impact on serum cytokine levels (TNF-α, IL-6, CRP), biochemical markers of organ function (AST, ALT, urea, creatinine, albumin), and histopathological alterations in the lung, kidney, and liver. The findings are expected to provide new insights into the molecular basis of zinc and vitamin E synergy and their potential as adjunctive therapy for sepsis.

MATERIALS AND METHODS

Materials

The experimental materials comprised male Wistar rats and a Staphylococcus aureus bacterial strain (ATCC 25293). Additional laboratory supplies included nutrient agar medium, sterile Petri plates, disposable syringes (1 mL), and essential analytical instruments such as a spectrophotometer and an automated hematology analyzer. A McFarland 0.5 turbidity standard was used to prepare bacterial suspensions. Other supporting materials consisted of sterile saline, disposable gloves, animal cages, and water bottles. Commercial ELISA kits (Solarbio, China) were employed to quantify serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), procalcitonin (PCT), and C-reactive protein (CRP). For biochemical assessments, diagnostic kits for serum Aspartate Aminotransferase (AST), serum Alanine Aminotransferase (ALT), creatinine, and urea were procured from DiaSys Diagnostic Systems, Germany. The nutritional supplements used zinc and vitamin E (α-tocopherol) were obtained as standard generic products.

Animal Preparation

A total of thirty male Wistar rats, weighing approximately 250–300 g, were utilized in this study. The animals were housed under uniform laboratory conditions with a regulated temperature of 22 ± 2°C and an alternating 12-hour light and dark cycle. Standard rodent chow and water were provided ad libitum. All experimental procedures received ethical approval from the Animal Research Ethics Committee of Universitas Prima Indonesia (Ethical Approval No: 189/KEPK/UNPRI/VI/2025).

Staphylococcus aureus bacterial inoculum preparation

The *Staphylococcus aureus* strain (ATCC 25293) was obtained from the American Type Culture Collection and propagated on nutrient agar plates. The cultures were incubated at 37°C for approximately 18–20 hours to promote optimal bacterial growth. Following incubation, a bacterial suspension was prepared in sterile physiological saline, and its density was standardized to the 0.5 McFarland turbidity reference, corresponding to roughly 1.5×10^8 CFU/mL, as determined spectrophotometrically at 600 nm. Each experimental rat was then intraperitoneally injected with 1 mL of the prepared bacterial suspension[10].

Experimental Design

The animals were randomly assigned to seven experimental groups, with five rats in each group (n = 5), and each group received a specific treatment as follows:

a. Group 1 (Normal Control): Rats that did not receive Staphylococcus aureus injection and were not given any supplementation.

b. Group 2 (Negative Control): Rats that received Staphylococcus aureus injection but were not given any supplementation.

c. Group 3 (Positive Control) (Levofloxacin 45 mg/kg BW + Zinc 0.9 mg/kg BW + Vitamin E 250 mg/kg BW): Rats that received Staphylococcus aureus injection and were treated with antibiotics and combined supplementation of zinc and vitamin E.

d. Group 4 (Zinc 0.9 mg/kg BW + Vitamin E 250 mg/kg BW): Rats that received Staphylococcus aureus injection and were supplemented with zinc and vitamin E.

e. Group 5 (Zinc 1.8 mg/kg BW + Vitamin E 250 mg/kg BW): Rats that received Staphylococcus aureus injection and were supplemented with zinc and vitamin E.

f. Group 6 (Zinc 2.7 mg/kg BW + Vitamin E 250 mg/kg BW): Rats that received Staphylococcus aureus injection and were supplemented with zinc and vitamin E.

All treatments were given once daily for seven consecutive days following sepsis induction. Vitamin E (α-tocopherol) was given at 250 mg/kg BW, adapted from Atli et al. (2012), who used this dose with selenium to protect against sepsis-induced lung injury. Levofloxacin (45 mg/kg BW) served as a positive control due to its proven efficacy against *Staphylococcus aureus*[12], making it a strong comparator as complementary model along with Zinc and Vitamin E

Induction of Sepsis and treatment design

Sepsis was induced experimentally by intraperitoneal administration of *Staphylococcus aureus* at a concentration of 1.5×10^8 CFU/Ml[13]. Rats designated for the sepsis model (Groups 2–7) each received 1 mL of the bacterial suspension, whereas the normal control group (Group 1) was injected with 1 mL of sterile physiological saline to serve as a placebo. After a 48-hour period to allow systemic infection to develop, treatment interventions were initiated according to the respective protocols of each group. This delayed-treatment design was selected to more accurately simulate clinical conditions, where therapeutic interventions are typically administered after infection has progressed rather than at the time of pathogen exposure. Such an approach is widely utilized in experimental sepsis research to assess pharmacological efficacy under established inflammatory conditions. Following sepsis induction, the rats received daily oral doses of zinc, vitamin E, levofloxacin, or their designated combinations for seven consecutive days.

Sample Collection and Analysis

Twenty-four hours after the final dose administration, blood was obtained from the inferior vena cava of each rat under anesthesia to reduce physiological stress and ensure accurate collection. Using aseptic techniques, blood was drawn with sterile syringes to prevent sample contamination. The collected samples were subsequently analyzed for hematological and biochemical parameters, including a complete blood count (CBC)[14]. Additionally, Bacterial growth on nutrient agar plates was assessed semi-quantitatively based on visible colony density using a previously validated scoring system: no growth (−), sparse growth (+), moderate growth (++), and heavy growth (+++). Scoring was performed independently by two observers blinded to treatment

groups to reduce bias. Inflammatory mediators tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), procalcitonin (PCT), and C-reactive protein (CRP) were quantified using Commercial enzyme-linked immunosorbent assay (ELISA). In addition, hepatic function indicators (AST, ALT, and albumin) and renal biomarkers (creatinine and urea) were assessed to determine potential organ alterations after treatment[15].

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). All results are presented as mean ± standard deviation (SD). Differences among experimental groups were evaluated through one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify significant pairwise differences. A *p*-value less than 0.05 was regarded as the threshold for statistical significance[16].

RESULTS AND DISCUSSION

Blood Bacterial culture

The result of the blood bacterial culture can be seen in Table 1 and Figure 1, which clearly demonstrates a pronounced difference in bacterial growth patterns among the experimental groups. Bacterial growth was semi-quantitatively assessed. The placebo control group showed heavy growth (+++), whereas all treated groups demonstrated either no growth (–) or sparse growth (+), indicating effective bacterial suppression

This observation verifies the establishment of infection in untreated animals and the subsequent protective response in those receiving supplementation. The unchecked bacterial growth in the negative control group mirrors the pathophysiological cascade of sepsis, where *S. aureus* dissemination triggers an uncontrolled systemic inflammatory response accompanied by oxidative stress and cellular injury[17]. These findings are consistent with the established understanding that *S. aureus* bacteremia can rapidly progress into severe sepsis when innate immune mechanisms are overwhelmed.

Interestingly, the absence of colonies in the supplemented groups indicates that zinc and vitamin E contributed to the enhancement of host defense mechanisms. Zinc is a critical micronutrient that supports both the structural and catalytic functions of numerous immune-related enzymes. It promotes macrophage and neutrophil activity, enhances the integrity of epithelial barriers, and regulates inflammatory mediator production[18]. Through these mechanisms, zinc restricts bacterial replication and limits systemic dissemination. α-Tocopherol, on the other hand, serves as a lipid-soluble antioxidant that protects immune cells from reactive oxygen species (ROS)–induced damage, thereby preserving phagocytic and bactericidal efficiency during infection.

Table 1. Blood bacterial culture.

Group	Bacterial Growth
Normal Control	–
Placebo Control	+++
Complementary Antibiotic Control	–
Zinc 0.9 mg/kg BW + Vitamin E 250 mg/kg BW	–
Zinc 1.8 mg/kg BW + Vitamin E 250 mg/kg BW	–
Zinc 2.7 mg/kg BW + Vitamin E 250 mg/kg BW	–

Table 2. Leukocyte Count

Groups	Leukocytes (10^9/L) ± SD
Normal Control	4.47 ± 1.58*
Placebo Control	9.79 ± 0.89
Complementary Antibiotic Control	4.39 ± 1.36*
Zinc 0.9 mg/kg BW + Vitamin E 250 mg/kg BW	5.82 ± 0.28*
Zinc 1.8 mg/kg BW + Vitamin E 250 mg/kg BW	5.93 ± 0.31*
Zinc 2.7 mg/kg BW + Vitamin E 250 mg/kg BW	5.76 ± 0.43*

Notes: * = significantly different from the placebo control group (P≤0.05)

A substantial reduction in bacterial presence was noted in the group treated with the highest dose of zinc combination with vitamin E. This outcome implies a dose-dependent synergism, wherein zinc reinforces enzymatic antioxidant defenses such as superoxide dismutase (SOD), while α-tocopherol prevents lipid peroxidation and stabilizes cell membranes under oxidative stress[19,20]. The combined effect maintains immune cell integrity and optimizes bacterial clearance. Moreover, the group treated with levofloxacin alongside zinc and vitamin E exhibited complete inhibition of bacterial growth, further reinforcing the notion that antioxidant therapy can potentiate antibiotic activity. By mitigating oxidative stress and inflammatory tissue injury, these micronutrients may improve antibiotic penetration and enhance bactericidal efficacy.

Leukocyte Count

Leukocyte count serves as one of the fundamental indicators of systemic inflammation and immune activation during sepsis. In this study, considerable variation in leukocyte levels was observed among the experimental groups, reflecting the physiological response to *Staphylococcus aureus* infection and subsequent treatment. The quantitative findings are summarized in Table 2.

The placebo control group exhibited a pronounced leukocytosis, with a mean value of 9.79 ± 0.89 × 10^9/L, indicating an acute inflammatory state induced by sepsis. In contrast, the normal control group maintained a stable leukocyte count within the physiological range (4.47 ± 1.58 × 10^9/L). Notably, all treated groups including the antibiotic combination and zinc–vitamin E regimens showed a significant reduction in leukocyte levels compared with the untreated septic group ($p \leq 0.05$), suggesting that these interventions effectively mitigated infection-driven immune hyperactivation.

The decline in leukocyte counts among the treated rats highlights the anti-inflammatory and immunomodulatory potential of zinc and α-tocopherol. Zinc regulates the proliferation and apoptosis of leukocytes through modulation of NF-κB signaling, thereby preventing excessive cytokine expression and uncontrolled immune cell expansion[21]. Meanwhile, α-tocopherol exerts antioxidant protection by scavenging reactive oxygen species (ROS), stabilizing cell membranes, and preserving the viability of immune effector cells[22]. Together, these mechanisms contribute to restoring immune equilibrium and preventing further leukocyte infiltration into peripheral tissues.

Moreover, the groups receiving higher doses of zinc (1.8 and 2.7 mg/kg BW) combined with vitamin E exhibited leukocyte levels that approached those of the normal control group. This pattern suggests a dose-dependent effect in which zinc enhances antioxidant enzyme activity. These findings corroborate earlier studies reporting that zinc deficiency exacerbates leukocytosis and cytokine overproduction, whereas supplementation restores immune homeostasis[23]. Likewise, vitamin E has been shown to temper leukocyte activation by inhibiting oxidative bursts and modulating prostaglandin synthesis[24]. Taken together, the observed normalization of leukocyte counts reinforces the hypothesis that the combined administration of zinc and α-tocopherol provides a protective, dual-action effect controlling infection-induced hyperinflammation while supporting physiological immune function during sepsis recovery.

Liver and Kidney Function

Liver and renal biomarkers are key indicators of systemic organ integrity during sepsis. In this study, substantial biochemical variations

Table 3. Result of Biochemical in liver function

Groups	Serum AST (U/L) ± SD	Serum ALT (U/L) ± SD	Albumin (g/dL) ± SD	Creatinine (g/dL) ± SD	Urea (g/dL) ± SD
Normal Control	134, 50 ± 9,55*	44.4 ± 8.91*	3.3 ± 0.16*	0.75 ± 0.07*	25.6 ± 3.21*
Placebo Control	223,25 ± 14,45	44.2 ± 4.21	2.5 ± 0.35	1.34 ± 0.23	40.6 ± 3.65
Complementary Antibiotic Control	123 ± 5.71*	116.2 ± 21.90*	3.28 ± 0.19*	0.78 ± 0.05*	25.2 ± 2.86*
Zinc 0.9 mg/kg BW + Vitamin E 250 mg/kg BW	167 ± 30.31*	77.2 ± 22.61*	3.34 ± 0.30*	0.63 ± 0.06*	15.6 ± 2.70*
Zinc 1.8 mg/kg BW + Vitamin E 250 mg/kg BW	165 ± 16,83*	73 ± 23.18*	3.4 ± 0.16*	0.64 ± 0.06*	11.2 ± 2.86*
Zinc 2.7 mg/kg BW + Vitamin E 250 mg/kg BW	176.2 ± 24.95*	63 ± 25.07*	3.26 ± 0.21*	0.71 ± 0.14*	18.6 ± 4.34*

Notes: * = significantly different from the placebo control group (P≤0.05)

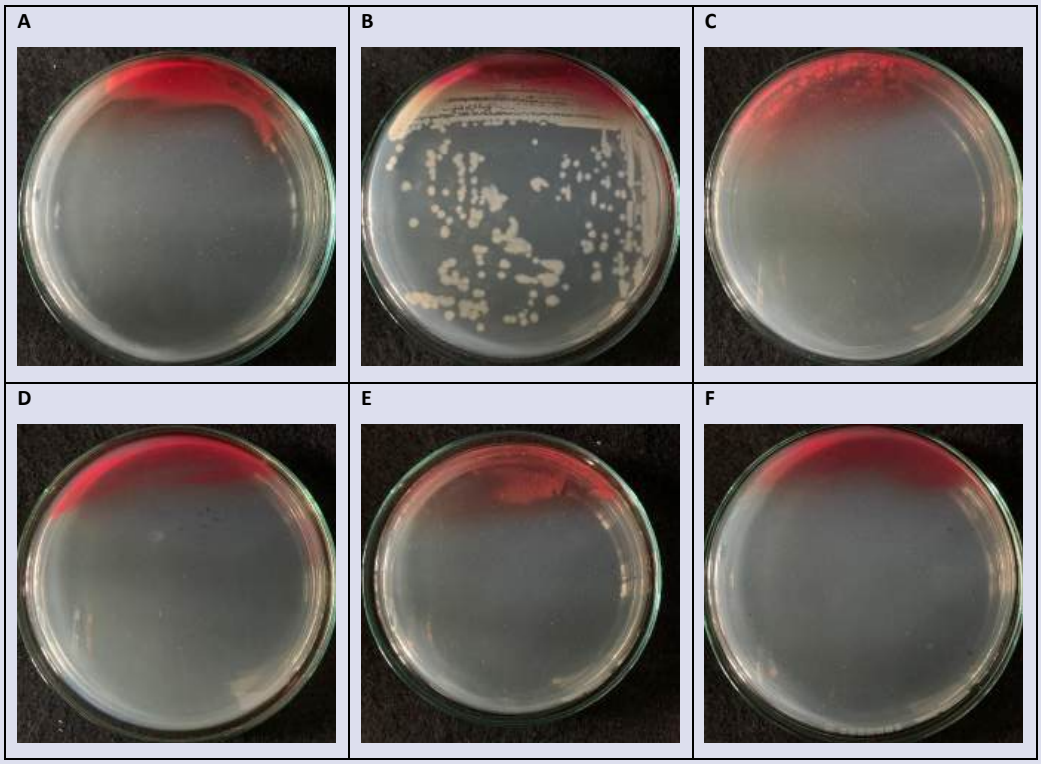

Figure 1. Blood bacterial culture: A) Group 1; B) Group 2; C) Group 3; D) Group 4; E) Group 5; F) Group 6.

were observed among groups, reflecting the extent of tissue injury and the protective efficacy of zinc and α-tocopherol supplementation. The summarized results are shown in Table 3.

The placebo control group exhibited markedly elevated levels of serum AST (223.25 ± 14.45 U/L) and ALT (144.2 ± 4.21 U/L), accompanied by decreased albumin and increased creatinine and urea concentrations. These alterations confirm the onset of hepatic and renal dysfunction induced by *Staphylococcus aureus* infection. Elevated transaminase activity is commonly associated with hepatocellular membrane disruption and oxidative stress, while the rise in urea and creatinine indicates renal impairment resulting from systemic inflammation and impaired filtration capacity.

Conversely, all treated groups demonstrated significant biochemical improvement ($p \leq 0.05$) compared with the untreated septic group. The normalization of AST and ALT levels in the zinc–vitamin E and antibiotic groups suggests effective hepatoprotection. Among these, the combination of zinc 1.8 mg/kg BW and vitamin E 250 mg/kg BW exhibited the most favorable response, with enzyme levels approaching those of the normal control group. Simultaneously, serum albumin levels increased significantly, reflecting restored hepatic synthetic function.

The hepatoprotective effect of zinc and α-tocopherol can be attributed to their complementary antioxidative and anti-inflammatory roles. Zinc stabilizes hepatocyte membranes by maintaining sulfhydryl groups in structural proteins and enhancing the activity of metallothionein, a potent free-radical scavenger[25]. It also modulates inflammatory pathways by inhibiting NF-κB activation, which reduces the production of hepatotoxic cytokines such as TNF-α and IL-6[18]. α-Tocopherol, in turn, neutralizes lipid peroxides within hepatocellular membranes, thereby preventing further enzymatic leakage and promoting cellular regeneration[26].

In terms of renal parameters, creatinine and urea levels decreased substantially in the supplemented groups compared to the placebo control. This suggests that the zinc–vitamin E combination alleviated renal oxidative burden and improved glomerular function. Zinc contributes to renal protection through its role in maintaining

antioxidant enzyme systems, while α-tocopherol prevents oxidative injury to renal tubular epithelium[27,28]. The improvement in these biochemical markers aligns with previous findings demonstrating that antioxidant micronutrients can mitigate sepsis-induced nephrotoxicity by preserving mitochondrial function and reducing lipid peroxidation. Overall, these findings indicate that zinc and α-tocopherol supplementation provides substantial hepatoprotective and nephroprotective effects in *S. aureus*-induced sepsis. Their synergistic interaction enhances antioxidant defense, suppresses inflammatory damage, and restores normal biochemical function, underscoring their potential as adjunct therapeutic agents in the management of septic organ injury.

Histological Evaluation of Kindey and Lung Tissues

Histopathological examination provides critical insight into the extent of cellular injury and the protective effects of therapeutic agents at the tissue level. Representative micrographs of kidney and lung sections from all experimental groups are illustrated in Figures 2 and Figure 3, respectively.

In the kidney tissues, the placebo control group exhibited marked pathological alterations characterized by glomerular necrosis, tubular epithelial degeneration, interstitial edema, and leukocyte infiltration. These morphological features are typical indicators of sepsis-associated acute kidney injury, where inflammatory mediators and reactive

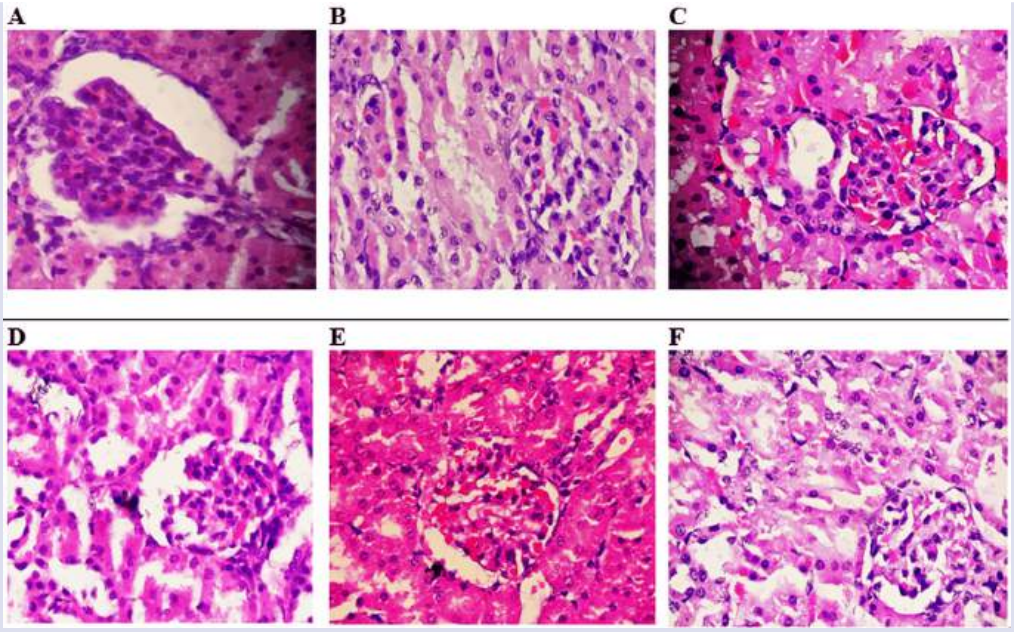

Figure 2. Histological Evaluation of Kidney Tissue. A) Group 1; B) Group 2; C) Group 3; D) Group 4; E) Group 5; F) Group 6.

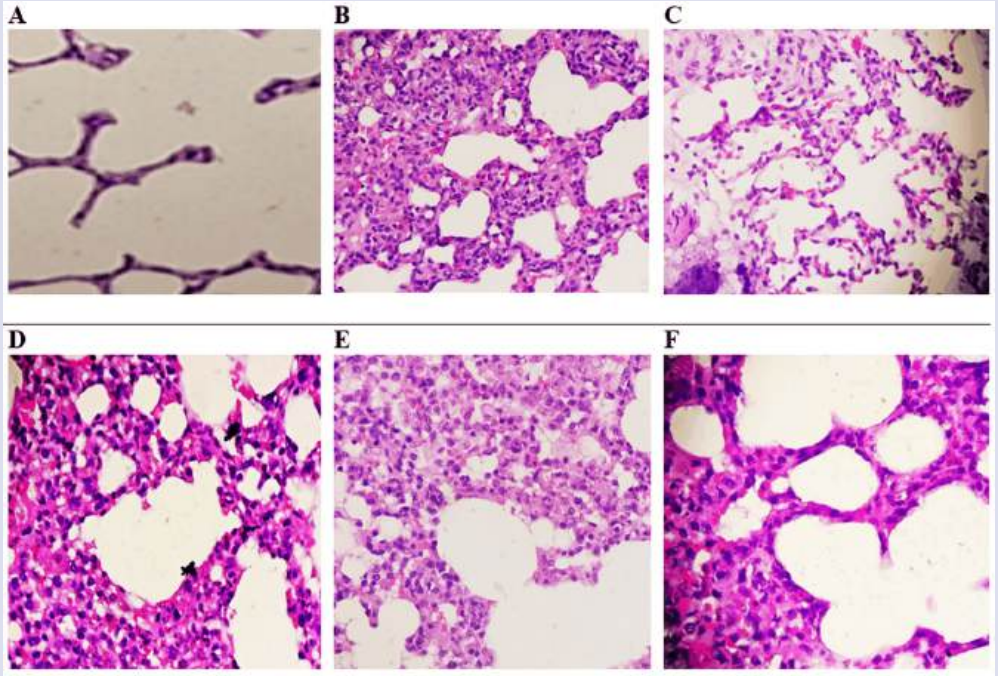

Figure 3. Histological Evaluation of Lung Tissue. A) Group 1; B) Group 2; C) Group 3; D) Group 4; E) Group 5; F) Group 6.

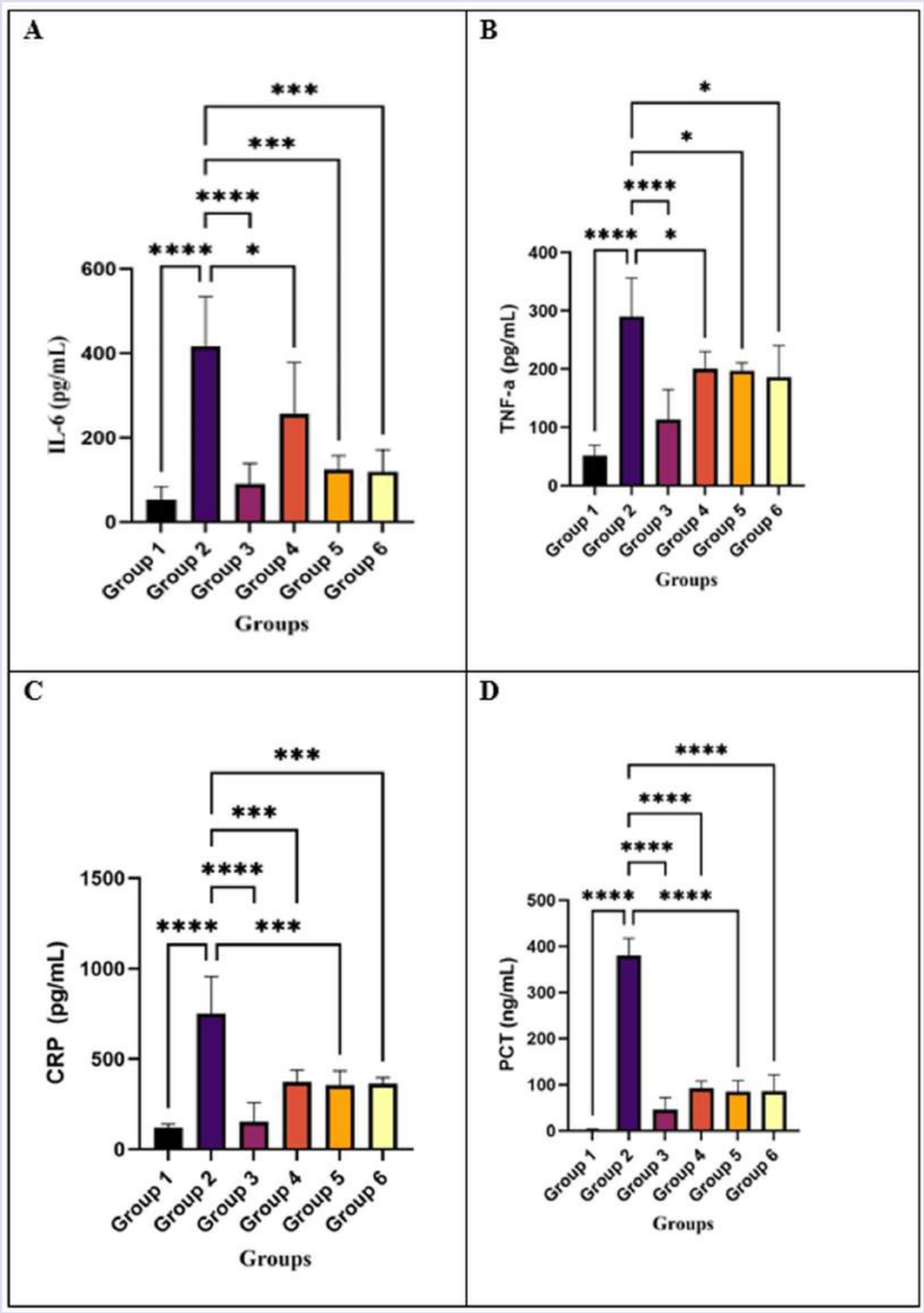

Figure 4. Cytokine marker levels. A) IL-6, B) TNF-α, C) CRP, D) PCT across treatment groups. Asterisk *P ≤ 0.05, **P ≤ 0.01, *** P ≤ 0.001, ****P ≤ 0.0001 represents significant differences to Placebo group and NS represents not significant (P > 0.05)

oxygen species (ROS) compromise renal microcirculation and induce oxidative damage to tubular structures[29]. In contrast, the normal control group maintained normal renal architecture with intact glomeruli and minimal interstitial changes.

Remarkably, all treatment groups displayed varying degrees of structural recovery. The kidneys of rats treated with zinc and α-tocopherol exhibited clear improvement in glomerular and tubular morphology, with reduced interstitial congestion and diminished inflammatory cell infiltration. The most notable restoration was observed in the group receiving zinc at 2.7 mg/kg BW combined with vitamin E at 250 mg/kg BW, which showed nearly normal histological architecture. This suggests that the combination therapy effectively prevented oxidative and inflammatory injury within renal tissues. The observed nephroprotective effect aligns with the biochemical results showing reduced serum urea and creatinine, indicating improved renal function. Mechanistically, zinc likely contributed to this protection through its role in maintaining antioxidant enzyme activity and stabilizing cellular membranes, while α-tocopherol attenuated lipid peroxidation and reduced endothelial dysfunction, both of which are hallmarks of sepsis-induced nephropathy.

Similarly, lung tissue examination revealed extensive inflammatory and structural damage in the placebo control group. The lungs showed severe alveolar collapse, thickening of alveolar septa, infiltration of polymorphonuclear cells, and areas of interstitial hemorrhage. These findings correspond to acute lung injury and developing acute respiratory distress syndrome (ARDS), commonly associated with sepsis[30]. In contrast, lung sections from the normal control group exhibited normal alveolar architecture with thin septa and no visible inflammatory changes.

Treatment with zinc and α-tocopherol significantly improved pulmonary histology. The groups receiving combination therapy showed reduced inflammatory infiltration, minimal hemorrhage, and restoration of alveolar structure. The highest protective response was evident in the high-dose zinc group (2.7 mg/kg BW), which displayed near-normal lung histoarchitecture with markedly decreased alveolar wall thickening. The improvement in pulmonary integrity supports the anti-inflammatory and antioxidative capacity of both agents. Zinc is known to regulate the expression of metallothionein and superoxide dismutase, thereby neutralizing ROS and limiting cytokine-induced endothelial permeability[25]. Meanwhile, α-tocopherol stabilizes cell membranes, inhibits lipid peroxidation in pulmonary tissue, and reduces neutrophil migration, collectively leading to the preservation of alveolar structure[26].

Taken together, the histopathological findings of the kidney and lung tissues corroborate the biochemical outcomes and confirm the protective efficacy of zinc and α-tocopherol against sepsis-induced multiorgan injury. Their synergistic action not only reduces oxidative and inflammatory damage but also promotes structural recovery at the tissue level. This evidence reinforces the hypothesis that combined zinc and vitamin E therapy serves as a potent adjuvant strategy to mitigate organ dysfunction and improve prognosis in sepsis.

Evaluation of Serum Inflammatory Cytokines

The measurement of proinflammatory cytokines offers a critical understanding of the systemic immune response during sepsis and the therapeutic impact of zinc and α-tocopherol administration. The comparative levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and procalcitonin (PCT) across treatment groups are shown in Figure 4.

A distinct pattern emerged across the experimental groups, illustrating that infection with Staphylococcus aureus markedly elevated the concentrations of all measured inflammatory mediators. The placebo control group exhibited the highest serum levels of IL-6, TNF-α, CRP, and PCT, confirming the onset of severe systemic inflammation following bacterial inoculation. These cytokines are well-known biomarkers of sepsis progression and serve as critical mediators in the "cytokine storm" that drives endothelial damage, oxidative stress, and organ dysfunction[31]. Elevated TNF-α and IL-6 initiate a cascade of inflammatory signaling, stimulating acute-phase reactants such as CRP and PCT, both of which correlate with infection severity and prognosis[32].

In contrast, all treated groups demonstrated a significant reduction in cytokine concentrations compared with the untreated septic rats ($p \leq 0.05–0.0001$), suggesting effective modulation of the inflammatory response. The normalization of cytokine levels was particularly evident in the group receiving the highest zinc dose (2.7 mg/kg BW) combined with vitamin E (250 mg/kg BW), indicating a dose-dependent synergistic effect. The antibiotic control group also showed a marked decrease in cytokine production, yet the zinc–vitamin E combination achieved comparable suppression even in the absence of antibiotic co-administration. This highlights the potential of micronutrient supplementation to attenuate inflammation through endogenous regulatory pathways rather than direct antibacterial mechanisms.

Mechanistically, zinc and α-tocopherol exert their anti-inflammatory effects through complementary molecular pathways. Zinc acts as a crucial regulator of immune signaling by inhibiting nuclear factor kappa-B (NF-κB) activation[7], a transcription factor responsible for upregulating genes encoding TNF-α, IL-6, and other proinflammatory mediators. By suppressing NF-κB nuclear translocation, zinc effectively dampens the early inflammatory surge that characterizes septic responses. Furthermore, zinc stabilizes cellular membranes and enhances the function of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, thereby mitigating oxidative stress that exacerbates cytokine production[33].

α-Tocopherol, in turn, provides lipid-soluble antioxidant protection that complements zinc's enzymatic activity. It neutralizes reactive oxygen species within phospholipid membranes, preventing lipid peroxidation and maintaining the structural integrity of immune and endothelial cells. This action limits the release of damage-associated molecular patterns (DAMPs) that perpetuate cytokine release[34]. Moreover, α-tocopherol modulates the activity of protein kinase C (PKC) and other signaling molecules involved in leukocyte activation, further contributing to the downregulation of inflammatory mediators.

The observed reduction in CRP and PCT levels in treated groups further supports the systemic anti-inflammatory effect of the zinc–vitamin E combination. CRP synthesis in the liver is primarily stimulated by IL-6, and its decline reflects the effective suppression of upstream cytokine signaling[35]. Similarly, decreased PCT levels suggest reduced bacterial burden and diminished systemic immune activation, aligning with the bacterial culture findings that demonstrated minimal or no bacterial growth in treated rats[36]. Together, these biochemical and microbiological results provide consistent evidence that the combination therapy successfully interrupts the inflammatory cascade at multiple levels pathogen clearance, cytokine regulation, and oxidative stress reduction.

These findings corroborate previous reports indicating that zinc supplementation attenuates proinflammatory cytokine expression in septic animal models, while vitamin E enhances antioxidant capacity and tissue resilience. Another studies also reported that co-administration of zinc and α-tocopherol results in greater suppression of TNF-α and IL-6 than when either is administered alone, supporting the synergistic hypothesis demonstrated here[37,38]. The restoration of cytokine homeostasis observed in this study suggests that the combined supplementation not only modulates immune reactivity but also

facilitates recovery of organ function by preventing excessive oxidative and inflammatory damage.

CONCLUSION

This study demonstrated that combined zinc and α-tocopherol supplementation effectively attenuates Staphylococcus aureus–induced sepsis in rats. Co-administration significantly reduced TNF-α, IL-6, and CRP levels, improved liver and kidney biochemical profiles, and enhanced serum albumin concentration. Histological analyses confirmed reduced inflammatory infiltration and improved tissue integrity, particularly at the highest zinc dose (2.7 mg/kg BW) with vitamin E (250 mg/kg BW). These findings suggest that zinc and vitamin E act synergistically through antioxidant and anti-inflammatory mechanisms, protecting against sepsis-induced multiorgan damage. The combination shows strong potential as a safe, adjunctive therapy for managing systemic inflammation and oxidative stress in sepsis.

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