Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.009

RESEARCH ARTICLE

# Protective Effect of Cell Death and Immune Checkpoint Inhibitors in Experimentally Induced Endotoxin Sepsis in Animal Model

Aqsa Ahmad[1] and Muhammad Imran Arshad[2*]

[1] Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan; [2]Department of Epidemiology and Public Health, Faculty of Health and Pharmaceutical Sciences, University of Agriculture, Faisalabad, Pakistan.
*Corresponding author: drimranarshad@yahoo.com; m.arshad@qu.edu.sa

ARTICLE HISTORY (25-1104)

Received: November 15, 2025
Revised: December 24, 2025
Accepted: December 28, 2025
Published online: January 27, 2026

**Key words:**
Gene Expression
Lipopolysaccharide
Mice
Necrostatin-1
Sepsis

**ABSTRACT**

Sepsis is a multifactorial condition characterized by multiple organ failure and is often lethal in clinical settings. Effective therapeutics those target immune and inflammatory pathways in sepsis remained obscure. The present study aimed to evaluate the effect of cell death inhibitors (separate and combined) and the therapeutic potential of immune checkpoint inhibitors in endotoxin-induced sepsis. The lipopolysaccharide (LPS) was injected intra-peritoneally to both male and female BALB/c mice. Cell death inhibitors (i.e., Necrostatin-1, PJ34, Sibiriline) and immune checkpoint inhibitors (anti-PD1 and anti-CTLA4) were administered after 01 hour of LPS administration. The histopathological changes in tissue samples were observed by H&E staining, while TUNEL assay was performed to check apoptotic cells. qPCR was performed to assess the mRNA level of pro-inflammatory cytokines and tissue injury biomarkers were also evaluated. Current study results indicated that the gross pathological changes in kidney, spleen, and liver tissue induced by LPS was reduced by Nec-1 and PJ34 administration in mice. The levels of tissue injury markers (AST, ALT) were significantly ($P \leq 0.05$) higher in mice challenged with LPS in comparison to control mice. The expression levels of pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) were elevated in LPS challenged group compared to control mice which were decreased by cell death and immune checkpoint inhibitors. In conclusion, exogenous administration of cell death and immune checkpoint inhibitors showed protective effect in LPS-induced sepsis in mice by decreasing inflammatory markers and organ injury.

**To Cite This Article:** Ahmad A, Arshad MI, 2026. Protective effect of cell death and immune checkpoint inhibitors in experimentally induced endotoxin sepsis in animal model. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2026.009

## INTRODUCTION

Sepsis is a complex disease that is present in various forms due to the host's disrupted regulation in response to the infection (Arora *et al.,* 2023; Li *et al*., 2025). According to the World Health Organization (WHO) report, 48.9 million cases of sepsis and 11 million sepsis-associated deaths occurred across the globe. It can occur in individuals of all age groups; however older adults and immunocompromised individuals are at higher risk. Though sepsis is widely studied in humans, it also signifies a major clinical and economic problem in veterinary medicine. Endotoxemia or sepsis can cause quantifiable changes in hemodynamics, inflammation, and organ dysfunction in veterinary species such as livestock and companion animals. Due to their increased vulnerability to bacterial endotoxins and propensity for gastrointestinal conditions like colic and diarrhea, horses are especially prone to spontaneous sepsis (Blangy-Letheule *et al.,* 2023). In ruminants, endotoxemia is mostly caused by an imbalance between pro and anti-inflammatory cytokines caused by *Escherichia coli* and *Salmonella spp* (de Laforcade *et al.,* 2003). For instance, a canine model of LPS induced sepsis exhibited a sharp increase in pro-inflammatory cytokines i.e. tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6), offering an animal model of immune dysregulation (Song *et al.,* 2012). A porcine polymicrobial septic shock model in pigs replicated the hypotension, hyperlactatemia, and organ damage associated with human septic shock (Zurek-Leffers *et al.,* 2023). Recent research suggests that oxidative stress and mitochondrial dysfunction play a role in sepsis associated organ injury in animals, making them potential therapeutic targets for veterinary species (Rodríguez *et al.,* 2011).

The mechanism of pathogenesis of sepsis is highly complex. The initial acute response of host to invaded

microorganisms usually causes macrophages to ingest the pathogen and turn out a variety of pro-inflammatory cytokines i.e. IL-6, TNF-α, Interleukin-1 beta (IL-1β), and Interferon gamma (IFN-γ) which in turn cause cytokine storms and activate the innate immune system (Reddy *et al.,* 2024; You, 2025). The pathogen associated molecular patterns (PAMPs) of bacteria and damage associated molecular patterns (DAMPs) serves as a starting signal to cause infections and hence up-regulate the expression of inflammatory genes (Wang *et al.,* 2023; Xu *et al.,* 2025). Usually, necrosis and apoptosis are two mechanisms of cell death involved in the pathogenesis of disease (Xu *et al.,* 2021). Necroptosis shares similar death inducers as apoptosis do, involving tumor necrosis factor (TNF), TNF-related apoptosis inducing ligand (TRAIL), TLRs and Fas (Saeed *et al.,* 2019). It has been confirmed that necroptosis plays a major role in triggering sepsis. Therefore, inhibition of necroptosis by chemical inhibitors such as necrostatin-1 (Nec-1), (Zhang *et al.,* 2019; Liu *et al*., 2025) PJ-34, and sibiriline can serve as a possible therapeutic target (Le Cann *et al.,* 2017).

In addition to hyper inflammation, sepsis induces immune suppression through the activation of immune checkpoint pathways. Molecules like programmed cell death receptor-1 (PD-1), its ligand (PD-L1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) inhibit proliferation of T-cells and production of cytokines, contributing to immune exhaustion (Busch *et al.,* 2020; Zhong and Yin, 2023). Expression of PD-1/PD-L1 has also been found in canine and equine inflammatory disorders, supporting the translational potential of checkpoint-targeted therapies in veterinary species, according to recent comparative studies (Kocikowski *et al.,* 2024). In septic models, experimental blockade of these checkpoints has been shown to improve survival and restore immune function (Liu *et al.,* 2022; Wang *et al.,* 2023).

In an endotoxin-induced sepsis model, the present study investigated the immunotherapeutic potential of cell death inhibitors (Nec-1, PJ-34, and sibiriline) and immune checkpoint inhibitors considering the limitations of conventional antimicrobial and supportive therapies. The objective was to evaluate their individual and combined effects on inflammatory response, immune modulation, organ dysfunction, and survival outcomes.

## MATERIALS AND METHODS

**Ethical Approval :** The study was carried out in accordance with the guidelines set forth by the Institutional Bioethics Committee (IBC), University of Agriculture Faisalabad (UAF), Pakistan (No., 188/ORIC, dated as 17-01-2022) for experimental protocol and research. All efforts were carried out to reduce the suffering and pain to the animals and 3R principle of animal experimentation was followed.

**Study design:** Balb/C male and female mice (20-25g) were obtained from Lab Animal Facility of Institute of Microbiology, UAF and housed in the animal house of the Institute of Microbiology, UAF. The animals were kept in pathogen-free cages and allowed to acclimate to the laboratory environment for three days at 25℃. During the entire period, the mice were provided with food and water *ad libitum*. The animals were divided into nine groups (n=7 in each group) i.e. Vehicle control/PBS, LPS, LPS+Nec-1, LPS+PJ34 and LPS+Sib, LPS+anti-CTLA4, LPS+anti-PD1, LPS+anti-PD1+anti-CTLA4 and LPS+Nec-1+PJ34+Sib.

The LPS (Solarbio, China) was injected intraperitoneally to induce sepsis in all groups except vehicle control/ PBS at a dose rate of 20mg/kg in a volume of 200-µL/mice. After 1 hour of LPS, treatment Necrostatin-1, PJ34 and Sibiriline were administered at dose rate of 10mg/kg in a volume of 200-µL/mice. However, anti-PD1 and anti-CTLA4 were administered at dose rate of 50µg/mice in a volume of 50-µL/mice (Table 1). Mice were euthanized at 6 hours post-treatment for blood and organ collection.

**Blood and Organ collection:** After 6 hours, blood was collected through retro-orbital route for serum biochemistry and mice were sacrificed to collect organs i.e. liver, kidney and spleen for histopathological analysis, TUNEL assay and cytokine analysis. The blood samples were centrifuged at 1500-2000 rpm for 15 minutes to separate serum. Serum was then stored at -20℃ for further use.

**Serum Biochemistry:** Tissue injury markers in mice serum were evaluated by measuring the changes in creatinine, urea, bilirubin and Gamma-Glutamyl Transferase levels. Liver injury was evaluated by measuring aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in auto-analyzer by using commercial assay kit (Human Diagnostic Worldwide, Germany). Following the manufacturer's instructions, serum dilutions were prepared and absorbance was measured at 340nm wavelength.

**Histopathological Analysis and TUNEL assay:** Liver, Spleen and Kidney were preserved in 10% neutral buffered formalin, blocked, cut into 5 µm thick, and stained by hematoxylin and eosin (H&E) for histopathological analysis. The TUNEL assay (Elabscience, China) was performed to check apoptotic cells according to the manufacturer's guidelines. The TUNEL-positive cells were counted at 12 different microscopic fields.

**RNA extraction and cDNA synthesis:** Total RNA from Liver, Kidney and Spleen of all groups was extracted by TRIzol method (Invitrogen, USA). RNA was quantified by Nanodrop ND-1000, and RNA integrity was determined on agarose gel. cDNA was synthesized from extracted RNA using the RevertAid First Strand cDNA synthesis kit (ThermoScientific, USA) according to manufacturer's guidelines.

**Gene expression analysis by qPCR:** Total RNA was converted into complementary DNA (cDNA) where they served as a template for qPCR. The cDNA was diluted 10-fold and stored at -20℃ until use. Before using SYBR green master mix, template cDNA and oligo primers (TNF-α, IL-6, IL-1β and 18S) were thoroughly mixed. The reaction mixture (20µL) was then transferred to 96-well PCR plate (Nest), sealed with adhesive film and placed in qPCR machine (Bio-Rad, CFX Manager). Software version 2.2.2

**Table 1:** Experimental groups and treatment protocols in BALB/C mice

| Groups | Dose | Route | No. of mice | Time |
|---|---|---|---|---|
| Vehicle control/PBS | 100µL/mice | Intraperitoneal | 7 | 6 hours |
| LPS | 20mg/kg | Intraperitoneal | 7 | 6 hours |
| LPS+Nec-1 | 20mg/kg+10mg/kg | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+PJ34 | 20mg/kg+10mg/kg | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+Sib | 20mg/kg+10mg/kg | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+anti CTLA4 | 20mg/kg+50µg/mouse | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+anti PD1 | 20mg/kg+200µg /mouse | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+antiPD1+anti CTLA4 | 20mg/kg+50µg+200 µg | Intraperitoneal | 7 | 1 hour post LPS injection |
| LPS+Nec-1+PJ34+Sib | 20mg/kg+10mg+10mg+10mg | Intraperitoneal | 7 | 1 hour post LPS injection |

of CFX Manager TM was used for the result analysis and fold change of mRNA expression was calculated by $2^{-\Delta\Delta CT}$ method.

**Statistical analysis:** The results were representative of experimental groups. One-way analysis of variance (ANOVA) was used for multiple group analysis and to find specific pairwise comparisons with significant mean differences, Tukey's test was performed, using GraphPad Prism 5 software. Statistical significance was defined as, *P<0.05, **P<0.01, and ***P<0.001.

## RESULTS

**Post-treatment with cell death and immune checkpoint inhibitors reduced elevated levels of tissue injury markers associated with LPS-induced sepsis:** To examine the kinetics of organ injury i.e. liver, kidney and spleen, BALB/c mice were intraperitoneally challenged with LPS. The gross anatomical examination revealed injury of liver, kidney and spleen in LPS challenged group in comparison to the control group. Gross changes noticed include splenomegaly, swollen kidneys and Liver along with discoloration in LPS challenged group (as shown in Fig. 1). The PBS control group showed normal architecture of organs.

Liver and kidney injury following LPS- induced sepsis was evaluated by measuring levels of ALT, AST, Creatinine, Urea, Bilirubin and GGT. All these levels were significantly elevated at 6 hours of post-LPS challenge in mice compared to the control group (Fig. 2), representing acute hepatocellular damage, kidney dysfunction, and

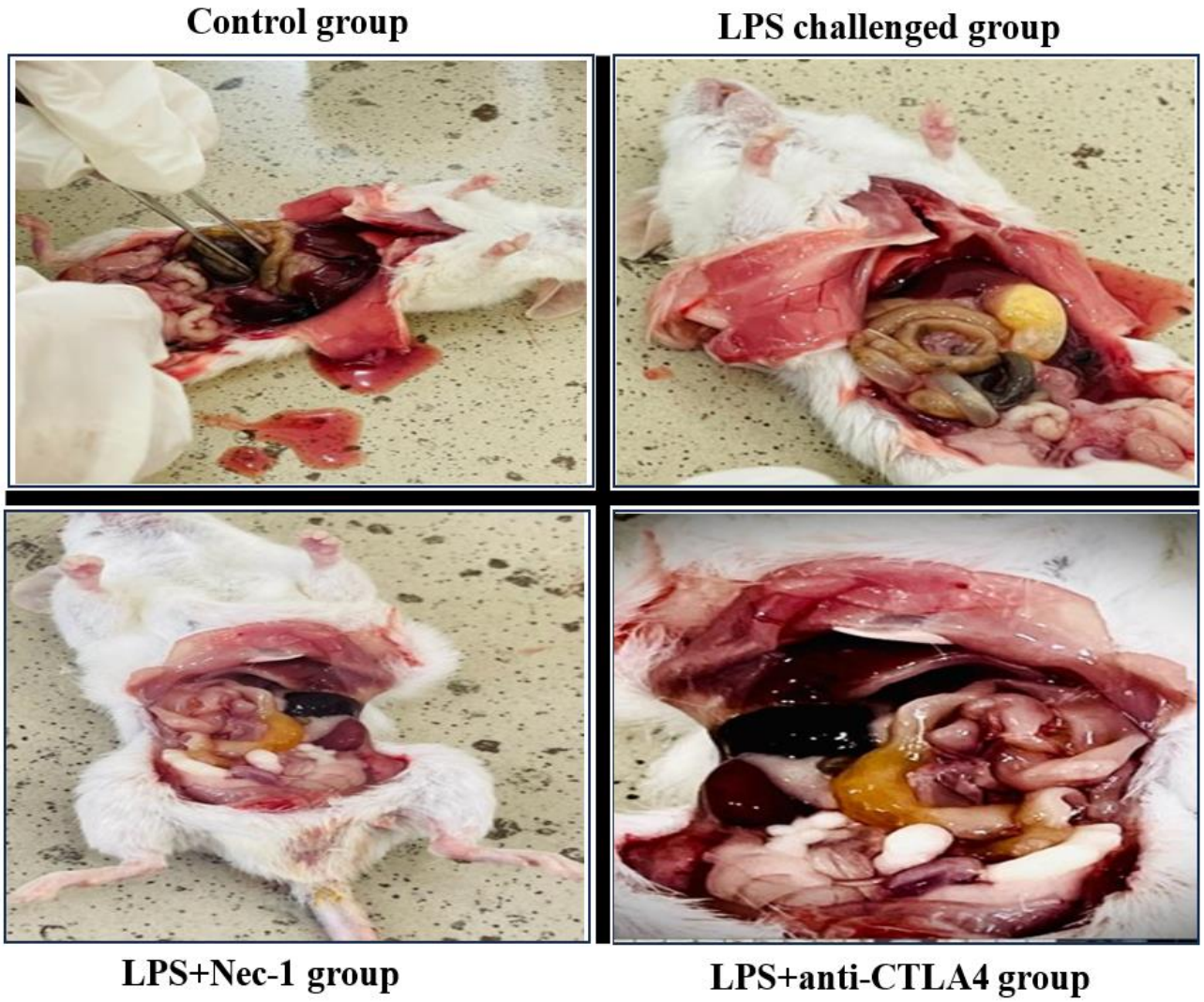


**Fig. 1:** Macroscopic examination of Spleen, Liver and Kidneys in BALB/c mice at 6 hours after LPS administration, cell death and immune checkpoint inhibitors to observe physical changes.

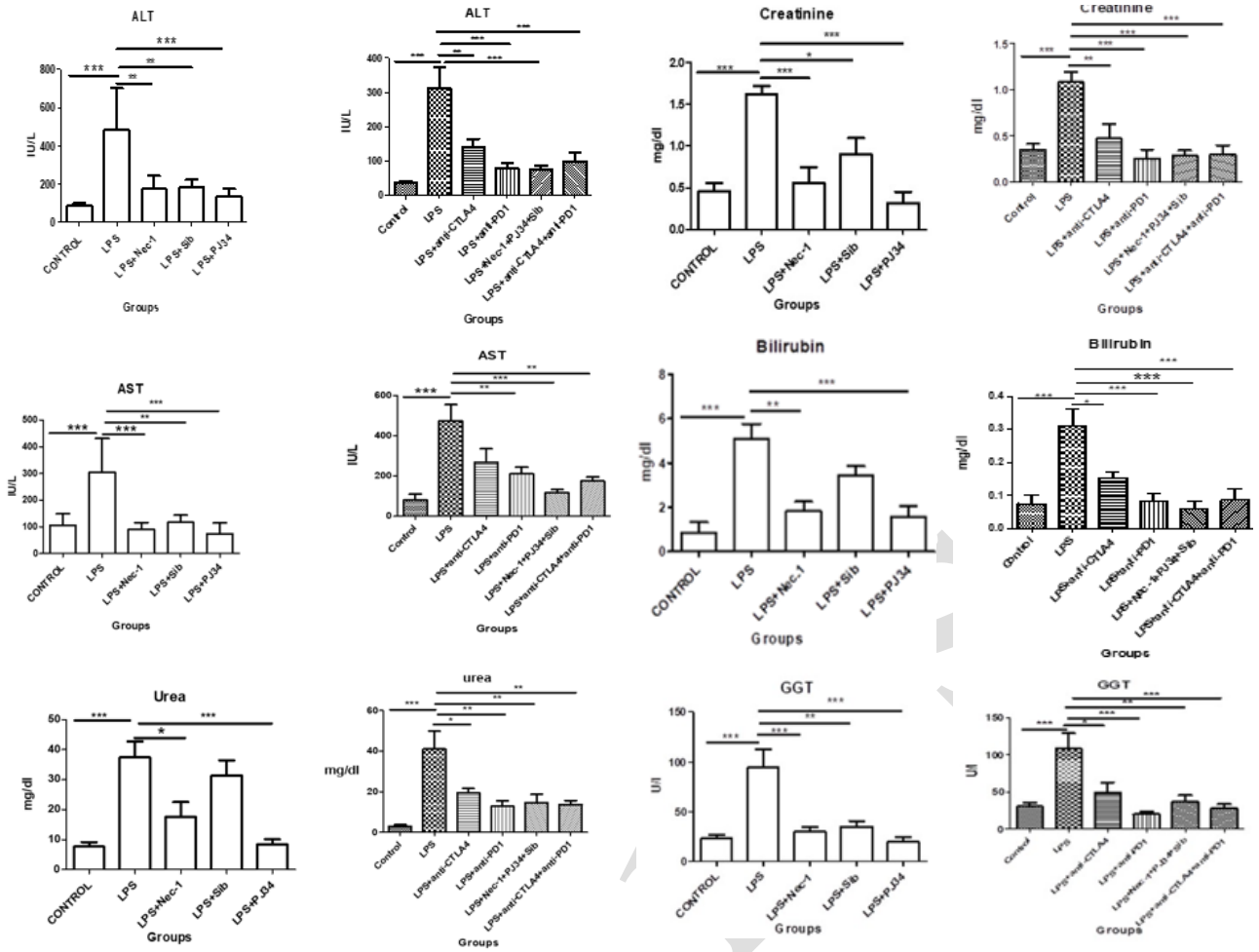


**Fig. 2:** Graphical representation of levels of tissue injury biomarkers measured at 6 hours after challenged with LPS and post treated with Necrostatin-1, Sibiriline, PJ34, anti-PD1, anti-CTLA4, Necrostatin-1 +PJ34+Sibiriline and anti-CTLA4+ anti-PD1. Data was analyzed by one-way ANOVA and Tukey's test.

impaired functions of liver. Treatment with cell death inhibitors *i.e.* (Nec-1, PJ34 and Sibiriline) effectively reduced these injury markers; however, PJ34 consistently showed the strongest protection among individual inhibitors. Furthermore, immune checkpoint inhibitors also contributed to reducing these markers, with anti-PD1 and combined cell-death inhibitor regimen (Nec-1+PJ34+Sibiriline) producing the most significant decrease, while anti-CTLA4 and combined immune checkpoint inhibitors showed moderate improvement. Statistical analysis showed that both treatment approaches significantly lowered LPS- induced kidney and liver damage, emphasizing the therapeutic potential of cell death and immune checkpoint inhibitors in mitigating sepsis-associated tissue injury.

**Histological evaluation and TUNEL analysis of liver, kidney and spleen in BALB/c mice following LPS induced sepsis and post treatment with cell death and immune checkpoint inhibitors:** The histopathological analysis of liver (Fig. 3a), Kidney (Fig. 3b) and Spleen (Fig. 3c) showed marked injury following LPS administration; however, mice in control group showed normal hepatic, renal and splenic architecture. LPS injected mice exhibited severe acute lesions in all organs, involving hepatocellular swelling, hemorrhage, degeneration and dense inflammatory infiltration in the liver; glomerular increase, tubular degeneration, inflammatory infiltration and congestion in the kidney; and pronounced extra-medullary hematopoiesis with disordered splenic architecture in the spleen. Treatment with Nec-1 and PJ34 constantly decreased organ injury to mild levels, whereas sibiriline showed less protection and showed moderate to severe pathological features. Groups treated with anti-PD1 and anti-CTLA4 effectively retain normal architecture of tissues and reduced inflammation in all organs. Combined immune checkpoint inhibitors *i.e.* (anti-PD1+ anti-CTLA4) showed more benefits, resulting in intact hepatic cords, normal glomeruli with fewer changes in tubules and normal follicles in spleen. Likewise, combined cell death inhibitors *i.e.* (Nec-1+PJ34+Sibiriline) significantly decreased the LPS-induced damage, limiting kidney and liver injuries to focal degeneration or mild congestion and complete preservation of splenic architecture.

To determine the sepsis-induced apoptotic changes in spleen (Fig. 4a), liver (Fig. 4b) and kidney (Fig. 4c) tissues of BALB/c mice, TUNEL assay was performed. Apoptotic cells were counted in 12 microscopic fields and mean/average number of apoptotic cells were counted manually in each treatment group. The results revealed that LPS treatment increased the numbers of TUNEL positive nuclei at 6 hours, which were then reduced by cell death and immune checkpoint inhibitors. The most significant decrease was observed by Nec-1, PJ34, anti-PD1, Nec-1+Sibiriline+PJ34 and anti-PD1+anti-CTLA4 treated as exhibited in (Fig. 5).

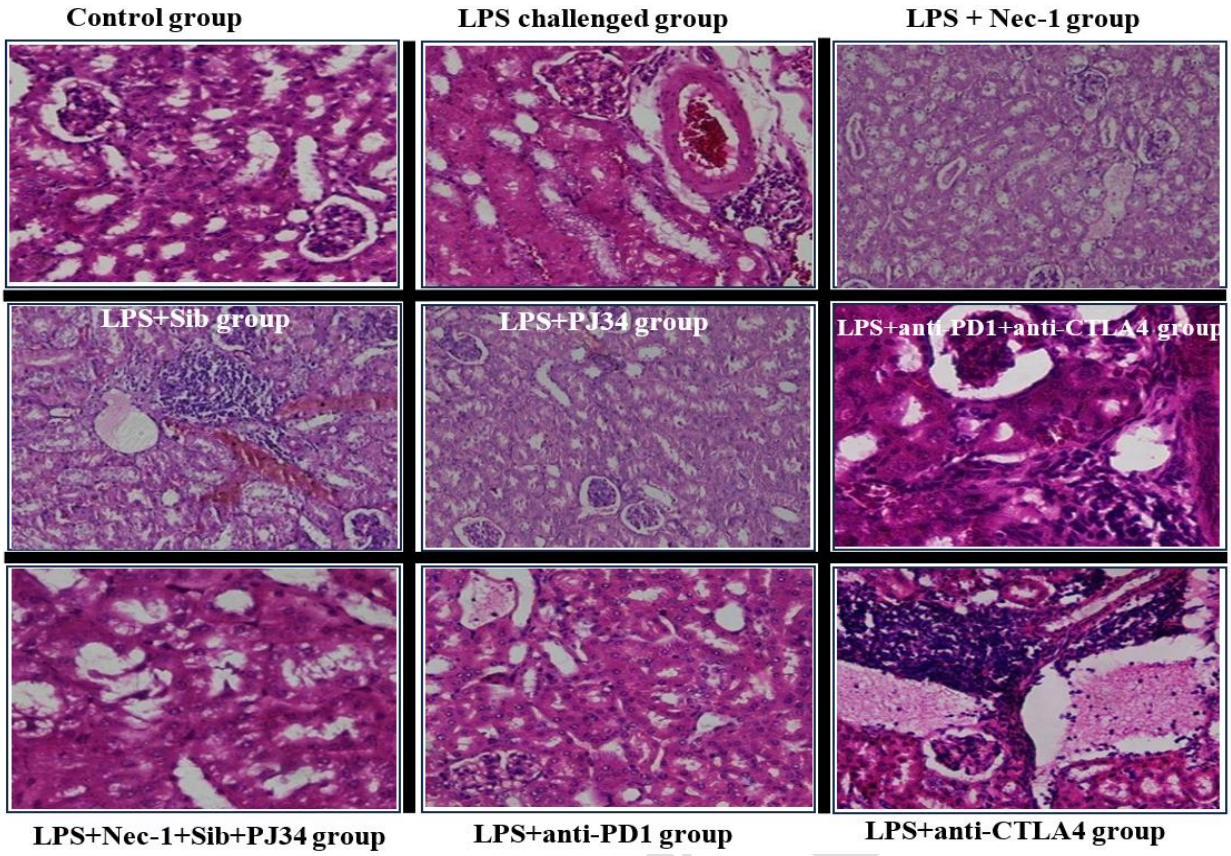


**Fig. 3a:** H&E staining of Liver section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, ecrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups at 100X.

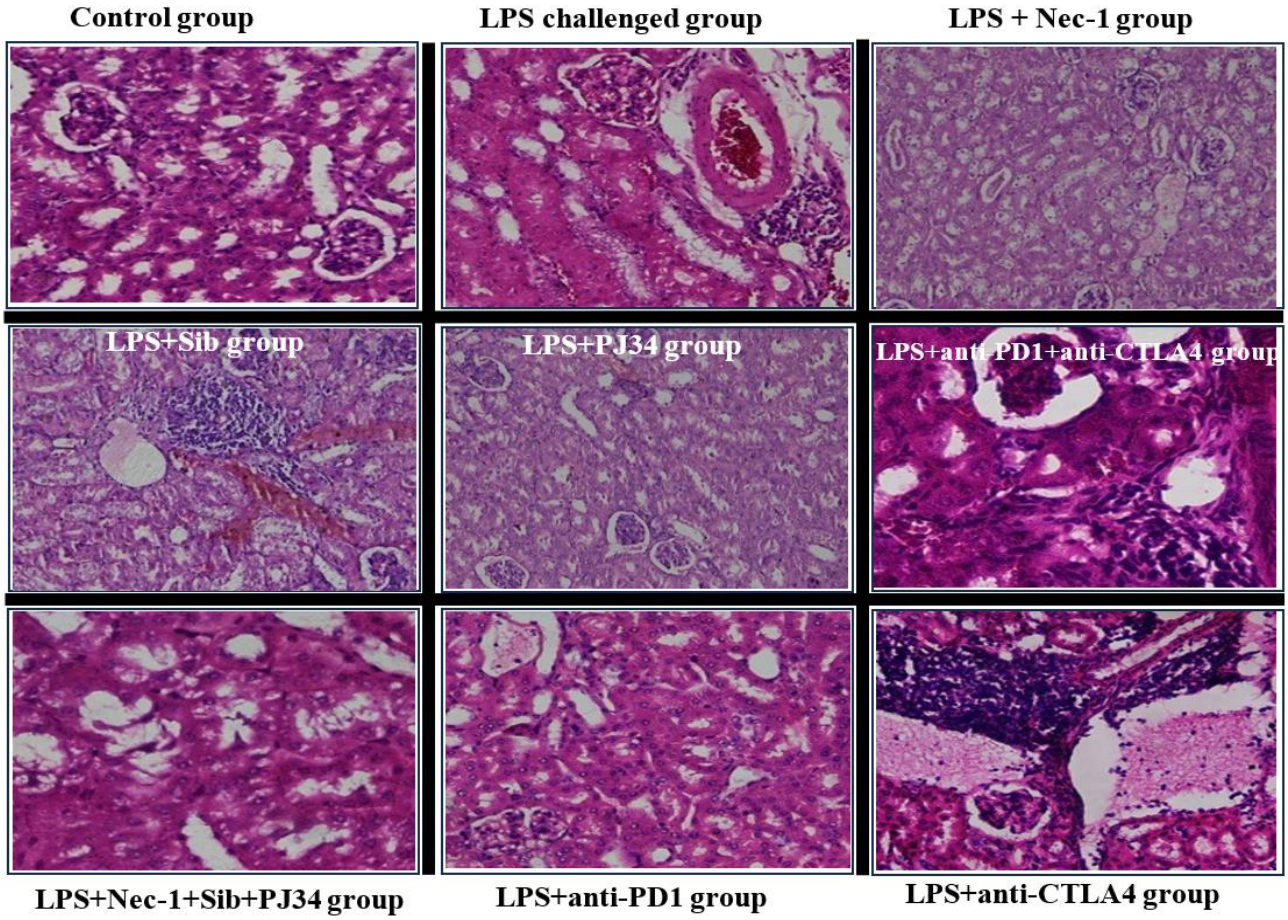


**Fig. 3b:** H&E staining of kidney section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, Necrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups at 100X.

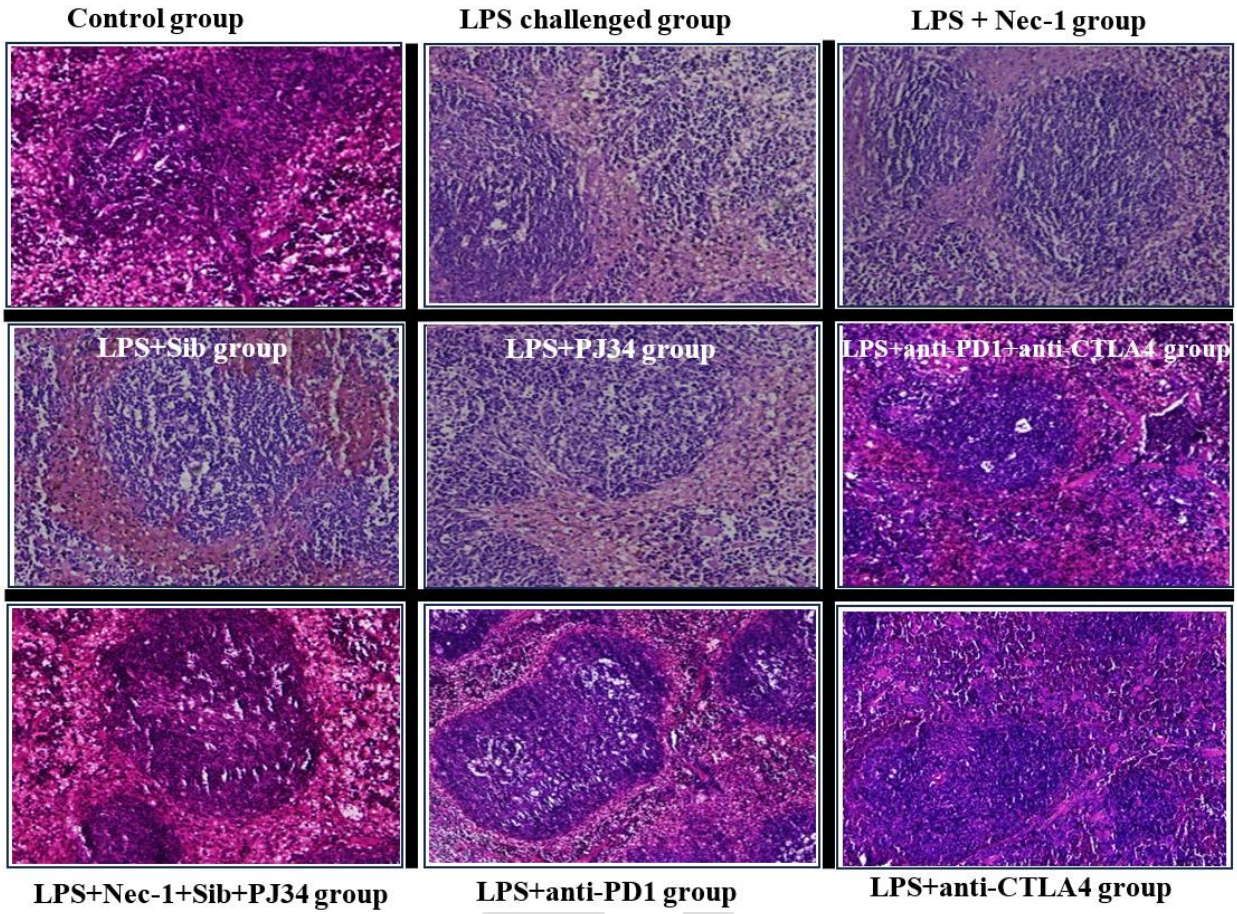


**Fig. 3c:** H&E staining of spleen section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, Necrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups at 100X.

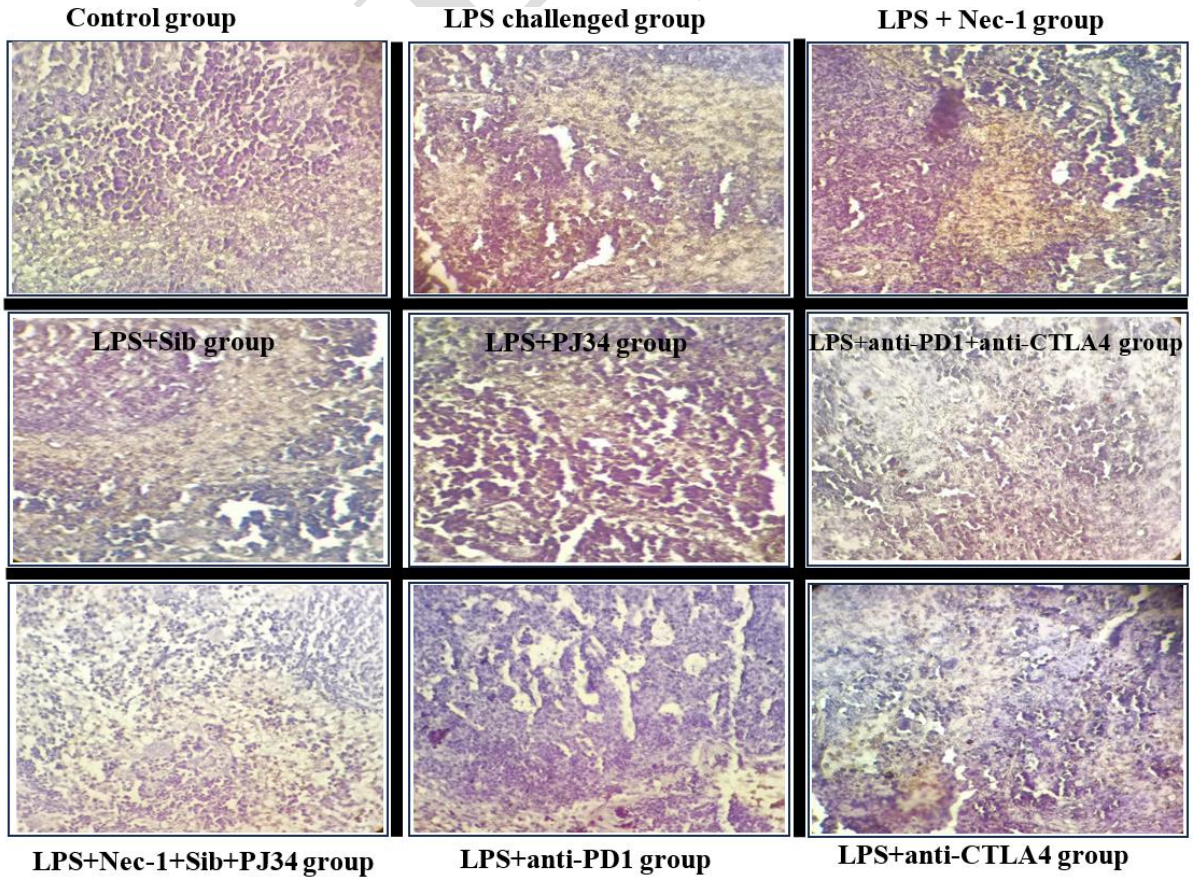


**Fig. 4a:** TUNEL assay to observe the apoptosis of Spleen tissue section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, Necrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups (400X).

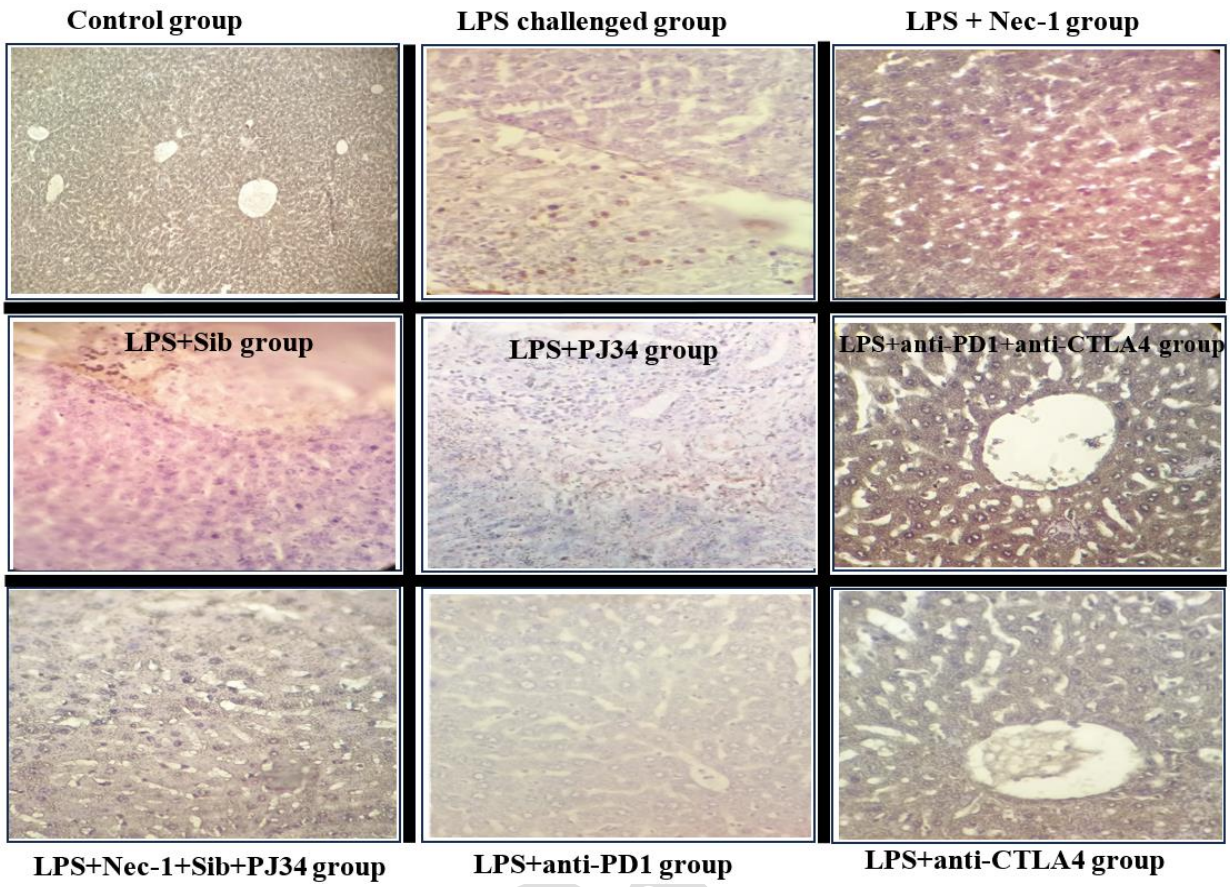


**Fig. 4b:** TUNEL assay to observe the apoptosis of Liver tissue section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, Necrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups (400X).

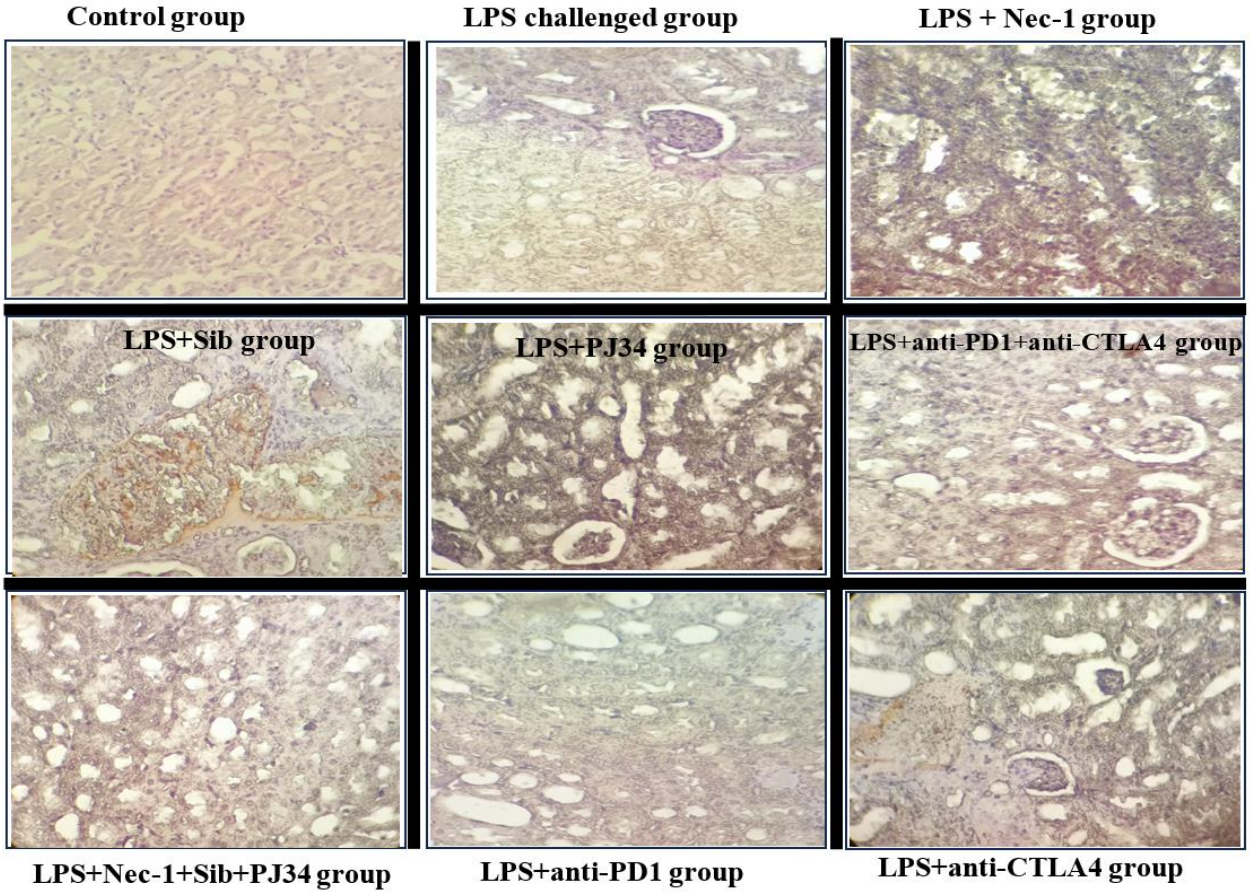


**Fig. 4c:** TUNEL assay to observe the apoptosis of Kidney tissue section in LPS induced sepsis mice model followed by Necrostatin-1, Sibiriline, PJ34, anti-PD1+ anti-CTLA4, Necrostatin-1+Sibiriline+PJ34, anti-PD1 and anti-CTLA4 treated groups (400X).

**Transcriptional expression of Pro-inflammatory cytokines in liver, kidney and spleen in BALB/c mice following LPS induced sepsis and post treatment with cell death and immune checkpoint inhibitors:** Cytokine mRNA expressions of IL-6, TNF-α and IL-1β were examined by RT-PCR on the liver, kidney and spleen tissues obtained from BALB/c mice in control, LPS challenged and treatment groups. As expected, systemic LPS administration induced a vigorous inflammatory response. At 6 hours post LPS injection, mRNA expression of the cytokines such as IL-6, TNF-α and IL-1β were all elevated in the liver, kidney and spleen as compared to the control group. However, groups treated with cell death and immune checkpoint inhibitors (individually or combined) caused significant suppression of mRNA expression of these pro-inflammatory cytokines in each treatment group. The more significant decrease was shown by Nec-1, PJ34, anti-PD1, Nec-1+Sib+PJ34 and anti-PD1+anti-CTLA4 treated groups as shown in (Fig. 6). These groups exhibited the greatest overall down regulation of cytokines across all organs evaluated. These findings showed that both cell death inhibition and immune checkpoint modulation effectively mitigate LPS induced inflammatory gene expression, with treatments producing strong anti-inflammatory effects.

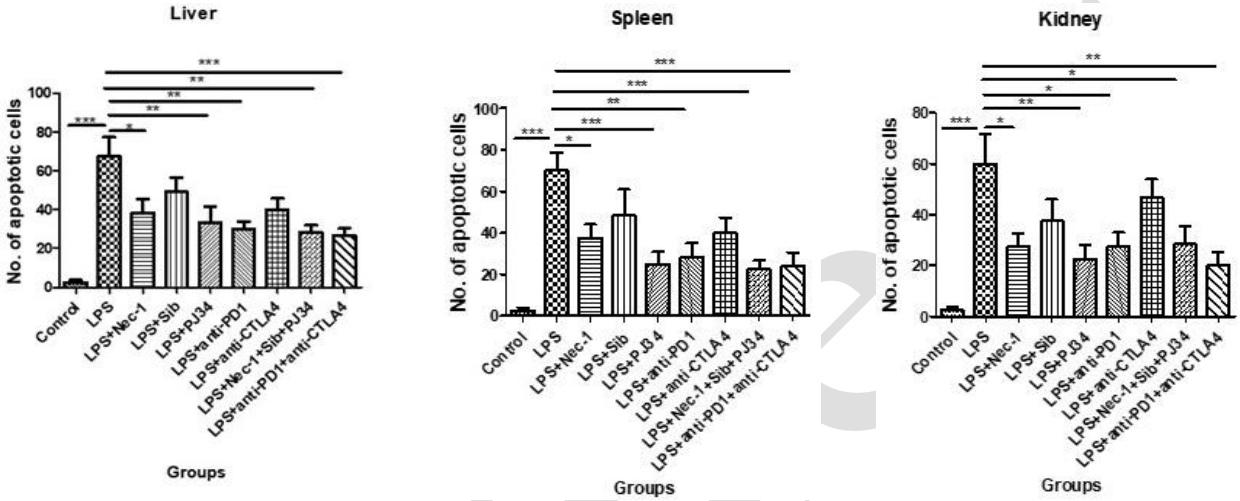


**Fig 5**. Analysis of Apoptotic cell frequency in Liver, Spleen and Kidney tissue of BALB/c mice following LPS induced sepsis and post treatment with cell death and immune checkpoint inhibitors.

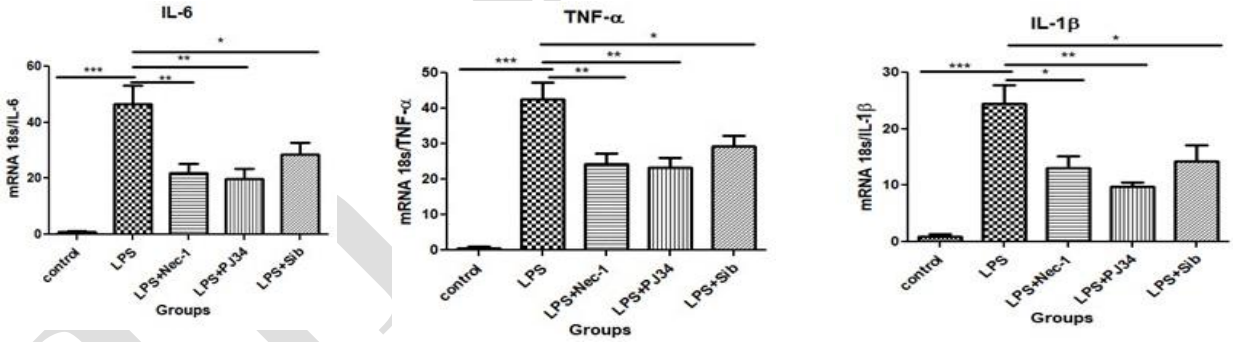


**Fig. 6a:** Relative fold change in mRNA expression of IL-6, TNF-α and IL-1β in liver tissue of LPS-induced sepsis mice model treated with Necrostatin-1, Sibiriline and PJ34 1-hour post injection. Data was analyzed by One-way ANOVA and Tukey's test. The PBS treated mice/control served as a reference for mRNA expression.

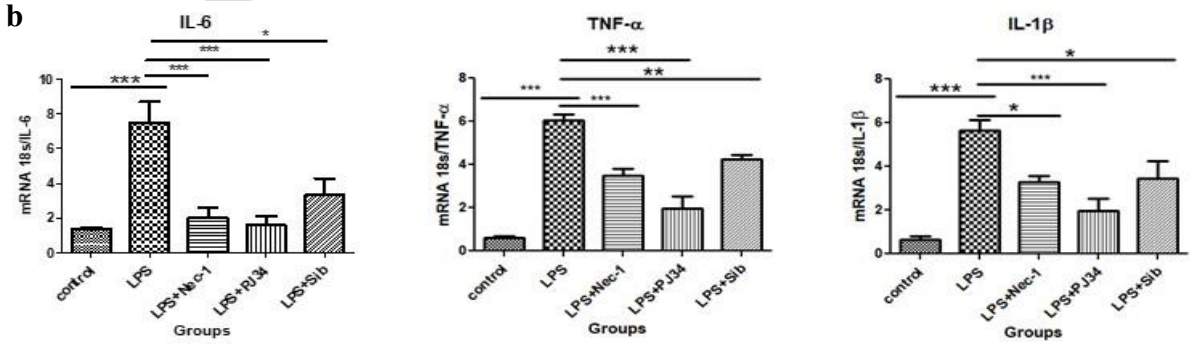


**Fig. 6b:** Relative fold change in mRNA expression of IL-6, TNF-α and IL-1β in kidney tissue of LPS-induced sepsis mice model treated with Necrostatin-1, Sibiriline and PJ34 1-hour post injection. Data was analyzed by One-way ANOVA and Tukey's test. The PBS treated mice/Control served as a reference for mRNA expression.

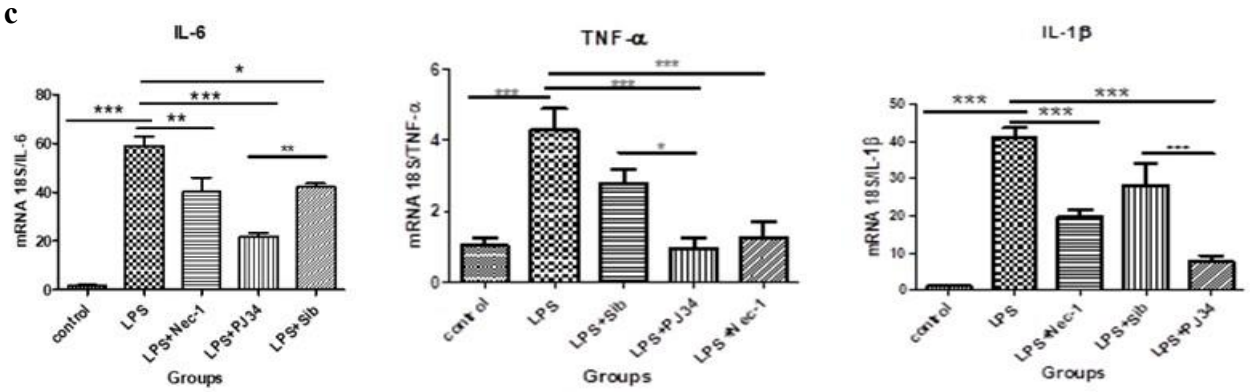


**Fig. 6c:** Relative fold change in mRNA expression of IL-6, TNF-α and IL-1β in spleen tissue of LPS-induced sepsis mice model treated with Necrostatin-1, Sibiriline and PJ34 1-hour post injection. Data was analyzed by One-way ANOVA and Tukey's test. The PBS treated mice/Control served as a reference for mRNA expression.

## DISCUSSION

Sepsis is a multifactorial syndrome characterized by dysregulated host response to infection, leading to multi-organ dysfunction such as cardiac, renal and hepatic injury. Despite ongoing advancements in understanding pathophysiology and organ dysfunction due to sepsis, no treatment specific for sepsis has yet been available (Huo *et al.,* 2023; Zhao *et al.,* 2022). The present study evaluated the immunotherapeutic potential of cell death inhibitors (Nec-1, PJ34 and Sibiriline) and immune checkpoint inhibitors (anti-PD1, anti-CTLA4) in an endotoxin induced murine model of sepsis.

Following LPS administration, histopathological and biochemical markers confirmed progressive hepatic and renal injury along with significant up-regulation of pro-inflammatory cytokines (IL-6, IL-1β and TNF-α). Treatment with Nec-1; significantly reduced serum urea and creatinine, consistent with early findings where Nec-1 mitigates kidney damage (Dong *et al.,* 2018). Likewise, literature indicated that pharmacologically inhibiting or blocking PARP enhanced renal function and histology by suppressing oxidative damage and production of pro-inflammatory cytokines (Tasatargil *et al.,* 2008). Despite being a potent RIPK1 inhibitor mechanically, Sibiriline produced a less pronounced biochemical improvement, indicating dose or time dependent effects as previously suggested (Cui *et al.,* 2023).

Elevated ALT and AST levels in hepatic tissue indicated liver damage caused by sepsis. These enzymes were significantly reduced by Nec-1 and PJ34 treatment, supporting the hypothesis that necroptosis contributes to hepatocellular injury in sepsis. (Fan *et al.,* 2016). Inflammation driven by hepatic damage that was mitigated by inhibiting necroptosis was further confirmed by LPS-induced cytokine overexpression and leukocytic infiltration.

In the second phase of this study, immune checkpoint inhibition was explored as an additional immunotherapeutic strategy. Administration of anti-PD1 alone or in combination with anti-CTLA4 and cell death inhibitors effectively reduced biochemical and histological markers of liver injury and inhibited the expression of inflammatory cytokines. These results are consistent with studies of improved survival in sepsis models following PD1 blockade (Zhu *et al.,* 2013). The benefits of anti-CTLA4 therapy were comparatively weaker, possibly because of the excessive immune activation caused by CTLA4 blockade (Lou *et al.,* 2023).

Spleen plays a significant role in regulating the immune system. Though, studies in animal models of sepsis had shown ample apoptosis of lymphocytes (Yuan *et al.,* 2023; Cao *et al.,* 2019) which may play a role in immune suppression and trigger sepsis results. Current study showed increase in gene expression levels of IL-6, TNF-α and IL-1β in LPS group which were then significantly reduced by anti-PD1 and combined treatment groups. However, no histopathological changes were developed except decrease in red pulp. These results were contrary to a study, in which LPS induced piglet model was used but the treatment was different. The pathological analysis in LPS group revealed swollen spleen, blunt edge and thick capsule. On histopathological examination it was recorded that white pulp volume was reduced (Xiao *et al.,* 2014).

These results suggest that targeting immune checkpoints and necroptosis together provides superior protection against endotoxin-induced organ injury. These molecular pathways may represent promising translational therapeutic targets due to the high prevalence of endotoxemia and sepsis in equine and canine patients. Further studies need to be carried out to explore dosage safety, specific responses and administration schedules to be recommended for clinical application choice both in human and veterinary medicine.

**Conclusions:** Investigation of advanced sepsis treatment methods is in demand to enhance treatment efficiency and to reduce sepsis associated mortality. This study evaluated the molecular response of various immunomodulatory sepsis treatments to provide basis for the development of personalized sepsis therapies. Results indicated that groups treated with PJ34, Nec-1, anti-PD1 were the most effective treatments revealing significant therapeutic potential in LPS- induced sepsis mice model. However, in contrast group treated with anti-CTLA4 showed less significant results. Histopathological analysis of kidney and liver tissue revealed inflammation and degeneration of hepatocytes, respectively. These findings suggest that anti-CTLA4 therapy is effective against tumor cells but in sepsis, it can have the opposite effect. Furthermore, combined cell death inhibitor therapy and immune-

checkpoint inhibitor therapy showed promising results in this study. As per our best knowledge, study regarding combined therapy is not reported yet. Hence, the current study addresses an important gap in understanding of sepsis treatment and offers a way for more therapeutic approaches that could enhance clinical outcomes.

**Acknowledgments:** The authors thank to the Ishtiaq Ahmed, Department of Pathobiology at Jhang campus of University of Veterinary and Animal Sciences, Lahore for helping us in histopathology.

**Authors Contributions:** MIA conceptualized the study. AA performed laboratory work and analyzed the results. MSM and AK reviewed the proof-read final version of the manuscript and helped in data analysis.

**Statements & Declarations**

**Funding:** All the authors declared no funding to disclose.

**Competing Interests:** The authors have no relevant financial or non-financial interests to disclose.

**Conflicts of Interests:** The authors declare that there are no conflicts of interest.

## REFERENCES

Arora J, Mendelson AA and Fox-Robichaud A, 2023. Sepsis: Network Pathophysiology and Implications for Early Diagnosis. Am J Physiol Regul Integr Comp Physiol 324:613–24.

Blangy-Letheule A, Vergnaud A, Dupas T, *et al.,* 2023. Spontaneous Sepsis in Adult Horses: From Veterinary to Human Medicine Merspectives. Cells 12:1-16.

Busch LM, Sun J, Cui X, *et al.,* 2020. Checkpoint Inhibitor Therapy in Preclinical Sepsis Models: A Systematic Review and Meta-Analysis. Intensive Care Med Exp 8:1–19.

Cao C, Yu M and Chai Y, 2019. Pathological Alteration and Therapeutic Implications of Sepsis-Induced Immune Cell Apoptosis. Cell Death Dis 10:1–14.

Cui N, Li S, Zhang Y, *et al.,* 2023. Discovery of Sibiriline Derivatives as Novel Receptor-Interacting Protein Kinase 1 Inhibitors. Eur J Med Chem 250:115190.

de Laforcade AM, Freeman LM, Shaw SP, *et al.,* 2003. Hemostatic Changes in Dogs with Naturally Occurring Sepsis. J Vet Intern Med 17:674-79.

Dong W, Li Z, Chen Y, *et al.,* 2018. Necrostatin-1 Attenuates Sepsis-Associated Acute Kidney Injury by Promoting Autophagosome Elimination in Renal Tubular Epithelial Cells. Mol Med Rep 17:3194–99.

Fan L, Li Z, Fan Z, *et al.,* 2016. The Effect of Necrostatin-1 On Expression of Liver Monocyte Chemotactic Protein-1 in Septic Rats. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 28:262–66.

Huo L, Liu C, Yuan Y, *et al.,* 2023. Pharmacological Inhibition of Ferroptosis As A Therapeutic Target for Sepsis-Associated Organ Damage. Eur J Med Chem 257:115438.

Kocikowski M, Dziubek K, Węgrzyn K, *et al.,* 2024. Comparative Characterization of Two Monoclonal Antibodies Targeting Canine PD-1. Front. Immunol 15:1-16.

Le Cann F, Delehouzé C, Leverrier-Penna S, *et al.,* 2017. Sibiriline, A New Small Chemical Inhibitor of Receptor-Interacting Protein Kinase 1, Prevents Immune-Dependent Hepatitis. The FEBS J 284:3050–68.

Liu H, Wang H, Zheng H, *et al*., 2025. Analyzing the Structure-Activity Relationship of Necrostain-1 and Its Analogues as RIPK1 Inhibitors on Necroptosis. Inter J Drug Dis Pharmacol 4:1-8.

Liu YC, Shou ST and Chai YF, 2022. Immune Checkpoints in Sepsis: New Hopes and Challenges. Int Rev Immunol 41:207–16.

Li Y, Ren S and Zhou SA, 2025. Advances in Sepsis Research: Insights into Signaling Pathways, Organ Failure, and Emerging Intervention Strategies. Exp Mol Pathol 142:1-12.

Lou Q, Gong J, Ye B, *et al.,* 2023. Acute Kidney Injury in Patients with Cancer Receiving Anti-PD-1/PD-L1 Antibodies: Incidence, Risk Factors, and Prognosis. Ren Fail 45:1–6.

Reddy H, Javvaji CK, Malali S, *et al.,* 2024. Navigating the Cytokine Storm: A Comprehensive Review of Chemokines and Cytokines in Sepsis. Cureus 16:1–10.

Rodríguez CC, Menge FW, Cerón JJ, 2011. Oxidative Stress in Veterinary Medicine. Vet Med Int 2011:01.

Saeed WK, Jun DW, Jang K, *et al.,* 2019. Necroptosis Signaling in Liver Diseases: An Update. Pharmacol Res 148:104439.

Song R, Kim J, Yu D, *et al.,* 2012. Kinetics of IL-6 and TNF-A Changes in A Canine Model of Sepsis Induced by Endotoxin. Vet Immunol Immunopathol 146:143-49.

Tasatargil A, Aksoy NH, Dalaklioglu S, *et al.,* 2008. Poly (ADP-Ribose) Polymerase as a Potential Target for the Treatment of Acute Renal Injury Caused by Lipopolysaccharide. Ren Fail 30: 115-20.

Wang L, Zhang Z, Chen X, *et al.,* 2023. Relationship Between the Expression of PD-1 And CTLA-4 On T Lymphocytes and the Severity and Prognosis of Sepsis. Int J Gen Med 16: 1513-25.

Wang M, Feng J, Zhou D, *et al.,* 2023. Bacterial Lipopolysaccharide-Induced Endothelial Activation and Dysfunction: A New Predictive and Therapeutic Paradigm for Sepsis. Eur J Med Res 28:1-16.

Xiao K, Qiu CW, Chen M, *et al.,* 2014. Distribution and Expression of Visfatin-Positive Cells in the Spleen of Lipopolysaccharide-Stimulated Piglets. Int J Morphol 32:1-7.

Xu Q, Guo J, Li X, *et al.,* 2021. Necroptosis underlies hepatic damage in a piglet model of lipopolysaccharide-induced sepsis. Front Immunol 12:1-18.

Xu Y, Wang J, Yuan R, *et al*., 2025. Targeting the Immuno-Inflammatory-Microbial Network: A Key Strategy for Sepsis Treatment. Front Immunol 16:1-15.

You W, 2025. Roles of Cytokine Storm in Sepsis Progression: Biomarkers, and Emerging Therapeutic Strategies. Front Immunol 16:1-5.

Yuan L, Wang Y, Chen Y, *et al.,* 2023. Shikonin Inhibits Immune Checkpoint PD-L1 Expression on Macrophage in Sepsis by Modulating PKM2. Int Immunopharmacol 121:1-11.

Zhang Q, Wei S, Lu J, *et al.,* 2019. Necrostatin-1 Accelerates Time to Death in a Rat Model of Cecal Ligation and Puncture and Massively Increases Hepatocyte Caspase-3 Cleavage. Am J Physiol Gastrointest Liver Physiol 316:551-61.

Zhang T, Yu-Jing L and Ma T, 2023. Role of Regulation of PD-1 and PD-L1 Expression in Sepsis. Front Immunol 14:1-12.

Zhao X, Zhang S and Shao H, 2022. Dexpanthenol Attenuates Inflammatory Damage and Apoptosis in Kidney and Liver Tissues of Septic Mice. Bioengineered 13:11625-35.

Zhong S and Yin Y, 2023. Regulatory Role of the Programmed Cell Death 1 Signaling Pathway in Sepsis-Induced Immunosuppression. Front Immunol 14:1-7.

Zhu W, Bao R, Fan X, *et al.,* 2013. PD-L1 Blockade attenuated Sepsis-Induced Liver Injury in A Mouse Cecal Ligation and Puncture Model. Mediators Inflamm 2013:1-7.

Zurek-Leffers FM, Lehmann F, Brabenec L, *et al.,* 2023. A Model of Porcine Polymicrobial Septic Shock. Intensive Care Med Exp 2023: 1-18.