Mehmet Akif Ersoy University Journal of Health Sciences Institute

MAKU J. Health Sci. Inst.
2025, 13(2): 1-11

Research Article

AMANTADINE MITIGATES LPS-INDUCED PANCREATIC INFLAMMATION VIA SUPPRESSION OF IFN-B, IL-1B, AND TNF-A

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ABSTRACT

Lipopolysaccharide (LPS)-induced systemic inflammation is known to impair pancreatic tissue integrity, particularly targeting β-cells within the islets of Langerhans, through cytokine-mediated injury. The research examined how amantadine (AMA) as an NMDA receptor antagonist protects against LPS-induced pancreatic damage in rats through histopathological and immunohistochemical assessments. The study involved thirty-two adult female Wistar albino rats weighing between 250–300 g which were randomly assigned to four groups (n=8): Control, LPS (5 mg/kg, i.p.), LPS+AMA (45 mg/kg, i.p.), and AMA-only. The rats received LPS administration followed by sacrifice under anesthesia six hours later. Pancreatic tissues were harvested for histopathological and immunohistochemical evaluations. Hematoxylin–eosin staining was used to assess edema, hyperemia, infiltration, and degeneration. Immunohistochemical analysis was performed to detect IFN-β, IL-1β, and TNF-α expressions in the islets of Langerhans. LPS administration resulted in significant histopathological injury, including interstitial edema, vascular congestion, inflammatory infiltration, and β-cell degeneration in the islets. Immunohistochemistry revealed a marked increase in IFN-β, IL-1β, and TNF-α expressions in the LPS group. Following AMA treatment both histopathological scores and cytokine expressions significantly mitigated compared to the LPS group ($p < 0.001$ for all). The control and AMA-alone groups maintained minimal expression of cytokine and normal pancreatic histology. LPS-induced pancreatic injury diminished via AMA treatment by suppressing inflammatory cytokine expression and preserving islet architecture. These findings suggest that AMA may have therapeutic potential in managing endotoxin-induced pancreatic dysfunction.

Key words: Amantadine, Lipopolysaccharide, Pancreatic inflammation, Islets of Langerhans, IFN-β, IL-1β, TNF-α, β-cell degeneration Immunohistochemistry, Rat model.

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Recevived: 03-06-2025

Revision request: 17-07-2025

Revision received: 18-07-2025

Accepted: 18-07-2025

Available online: 29-08-2025

INTRODUCTION

Sepsis represents a dangerous systemic inflammatory condition which develops from infections and results in multi-organ dysfunction that affects the pancreas. The pancreas shows susceptibility to inflammatory damage in its islets of Langerhans because of its extensive blood supply and its sensitive immunological nature (Dludla et al., 2023; Ozmen and Topsakal 2019; Ozmen and Topsakal 2022; Topsakal et al., 2024). Lipopolysaccharide (LPS), a component of Gram-negative bacterial membranes, is frequently used to model sepsis in

DOI: 10.24998/maeusabed.1713336

experimental settings and is known to provoke robust inflammatory responses by activating toll-like receptor 4 (TLR4) and downstream NF-κB signaling pathways (Akira et al., 2004; Wang et al., 2023; Chen et al., 2025).

LPS exposure leads to the overexpression of several pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon-beta (IFN-β), which play key roles in cellular dysfunction and apoptosis within pancreatic tissue (Lei et al., 2022). Previous studies have demonstrated that systemic inflammation can disrupt insulin secretion and β-cell viability through direct cytokine-mediated cytotoxicity (Eizirik et al., 2020). Furthermore, pancreatic β-cells exhibit limited antioxidant capacity, rendering them highly susceptible to cytokine-induced oxidative stress and inflammatory signaling cascades (Cnop et al., 2005; Ozmen and Topsakal 2019; Ozmen and Topsakal 2022).

The NMDA receptor antagonist AMA has established neuroprotective and antiviral properties but recent evidence shows its anti-inflammatory effects may also occur in peripheral organs (Jiménez-Jiménez et al., 2020; Costa, 2021). Research shows AMA reduces cytokine production while blocking inflammasome activation and reducing oxidative stress in models of neuroinflammation and ischemic injury (Xing et al., 2018; Sahin et al., 2023; Özmen et al., 2024). However, its potential protective role in extra-neural organs, particularly the pancreas, has not been fully elucidated.

Research conducted by Yang et al. (2024) demonstrates that NMDA antagonists such as AMA can reduce systemic cytokine cascades when used to treat peripheral inflammation. Given the overlap between pathways activated in neuroinflammation and those driving β-cell damage, there is a scientific rationale for investigating AMA in this context. Additionally, the islets of Langerhans share several molecular targets with CNS tissues, such as TLR4, NLRP3, and IFN-β-mediated signaling, further supporting this approach (Liu et al., 2023).

The aim of this study is to investigate the protective effects of the NMDA receptor antagonist AMA on the pancreas under conditions of sepsis and systemic inflammation induced by lipopolysaccharide (LPS). This was achieved through comprehensive histopathological evaluation and immunohistochemical analysis of key pro-inflammatory cytokines, including TNF-α, IL-1β, and IFN-β, within the islets of Langerhans. To the best of our knowledge, this represents one of the first experimental studies to explore the therapeutic potential of AMA beyond the central nervous system. The findings are particularly relevant for individuals receiving AMA therapy for neuroinflammatory disorders, as pancreatic lesions—often overlooked in the presence of neurological damage—may be preventable through such intervention, thereby reducing the risk of subsequent metabolic complications.

MATERIAL AND METHODS

Animals and Experimental Design

In this study, pancreatic tissues from rats used in a previous investigation that examined the effects of brain tissue were evaluated; no new animal experiment was conducted. During the original study, while the effects of LPS and AMA on other tissues were also being assessed, notable improvements were observed in the pancreatic tissues, prompting a decision to further evaluate them. Accordingly, approval was obtained from the Local Ethics Committee for Animal Research at Süleyman Demirel University for the publication of findings derived from the pancreatic tissues (Approval No: SDU HADYEK 555). Additionally, the research was supported by the Scientific Research Projects Unit of Süleyman Demirel University under project number TSG-2024-9515.

Thirty-two adult female Wistar albino rats weighing 250–300 g were obtained from the Experimental Animal Research Center at Süleyman Demirel University. The animals were kept in standard polycarbonate cages under controlled conditions (temperature 22 ± 2 °C, humidity 55 ± 5%, and a 12-hour light/dark cycle) with free access to standard rodent feed and water. After one week of acclimation, the rats were randomly assigned into four experimental groups (n = 8 per group) as follows:

1-Control group: Received intraperitoneal (i.p.) injections of 1 mL sterile physiological saline.

2-LPS group: Received a single i.p. dose of lipopolysaccharide (5 mg/kg, from Escherichia coli O111: B4; Sigma-Aldrich, USA) dissolved in 0.5 mL saline. (Húngaro et al., 2020).

3-LPS+Amantadine (LPS+AMA) group: Received amantadine hydrochloride (45 mg/kg, i.p.; Sigma-Aldrich, USA) 30 minutes prior to LPS administration.

4-Amantadine-only (AMA) group: Received a single i.p. dose of amantadine (45 mg/kg) without LPS (Danysz et al., 2021).

Six hours after LPS injection, all rats were anesthetized using a combination of ketamine (90 mg/kg,

i.p.) and xylazine (10 mg/kg, i.p.). Following midline abdominal incision and pancreatic tissues were excised. For histopathological and immunohistochemical examinations, the tissues were preserved in 10% buffered formalin.

Histopathological method

After euthanasia pancreatic tissues were harvested within ten minutes and they promptly fixed in 10% buffered formalin. The samples remained in the fixative for 48 hours before undergoing standard tissue processing using a fully automated tissue processor (Leica ASP300S, Leica Microsystems, Nussloch, Germany). Then, tissues were embedded in paraffin wax, and 5 µm-thick sections were prepared using a fully automated rotary microtome (Leica RM2155, Leica Microsystems, Nussloch, Germany). These sections were subsequently dried, deparaffinized, and rehydrated through a graded ethanol series. Hematoxylin and eosin staining was carried out, with Harris hematoxylin applied for five minutes and eosin (Tek-Path, Izmir, Türkiye) for two minutes. Following staining, the slides were mounted with coverslips and examined under a light microscope (Zeiss Axioscope 5 trinocular microscope, Carl Zeiss Microscopy GmbH, Jena, Germany). Within one week to ensure optimal tissue integrity the entire histological workflow—from fixation to staining—was completed.

The microscopic evaluation took place without knowledge of the sample identities. Histopathological assessment of the pancreatic tissue was performed in ten distinct fields per rat. Hyperemia, edema, and inflammation were individually graded on a semi-quantitative scale from 0 to 3, where 0 denoted the absence of pathology and 3 represented severe alterations. The number of damaged cells in each section received a score from 0 to 3 where 0 indicated no degenerated cells and 3 indicated more than eight degenerated cells. Each pancreatic sample was evaluated independently twice by the same experienced pathologist. Additionally, 100 Langerhans islets were randomly selected from each sample, and cell counting was performed using the counter tool in ImageJ software (National Institutes of Health, Bethesda, MD, USA), which allowed for marking cells with colored dots. The final histopathological score for each animal was determined by averaging the results of the two independent evaluations.

Immunohistochemical examination

From the previously prepared paraffin-embedded tissue blocks, four consecutive sections were cut and mounted onto poly-L-lysine-coated slides. Immunohistochemical staining was carried out using the streptavidin–biotin technique in accordance with the manufacturer's protocols, targeting the expression of interferon-β (IFN-β) [Anti-IFN-β antibody (ab140211)], interleukin-1β (IL-1β) [Anti-IL-1 beta antibody [RM1009]] (ab283818), and TNF-α [Anti-TNF alpha recombinant antibody [RM1005], ab307164]. The tissue sections were incubated with the primary antibodies, each diluted 1:100 in antibody diluent (ThermoFisher Scientific, MA, USA), for 60 minutes. The tissue sections treated a biotinylated secondary antibody and streptavidin–alkaline phosphatase complex after the primary antibody incubation period. The secondary detection was performed using a rabbit-specific HRP/DAB IHC Detection Kit—micro-polymer (ab236469, Abcam, Cambridge, UK). Sections were exposed to DAB for 3 to 5 minutes to visualize the immunoreactivity. For negative controls, the primary antibody was substituted with the antibody diluent. All immunohistochemical procedures were carried out in a blinded manner by an experienced pathologist from an external institution, who was unaware of the group allocations of the pancreatic samples. Each antibody was assessed independently.

The percentage of positively stained cells was assessed for each slide, and the overall proportion of positive cells was calculated for all groups. The count of positively stained cells per high-power field and the number of immunopositive cells per islet were measured and compared with the control group. Quantitative analysis of the immunostaining data was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Representative microphotographs were captured with the CellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) and subsequently subjected to statistical analysis.

Statistical analysis

A power analysis was conducted prior to the study using G*Power software (version 3.1.9.7) to estimate the minimum required sample size. Based on an effect size of 0.8, an alpha level (α) of 0.08, and a desired power (1–β) of 0.95, it was determined that seven rats per group would provide adequate statistical power.

The study presented descriptive statistics through medians and means with standard deviations and frequencies and percentages and minimum-to-maximum values based on the data type. The distribution and variance homogeneity of continuous variables were assessed using the Shapiro-Wilk, Levene, and

Kolmogorov-Smirnov tests. Variables with a normal distribution were reported as mean ± standard deviation, while non-normally distributed variables were expressed as medians with ranges.

For comparisons involving non-parametric data, the Kruskal-Wallis test and Mann-Whitney U test were used. When normality assumptions were met, one-way ANOVA followed by Tukey's post hoc test was applied. In the immunohistochemical analyses, the proportion of immunopositive cells was quantified. To evaluate the interaction effects between AMA and LPS exposure, a two-way ANOVA was performed. Statistical analyses were conducted using GraphPad Prism v10 software (GraphPad, San Diego, CA, USA), and a p-value of less than 0.05 was considered statistically significant.

RESULTS

Histopathological Findings in Pancreatic Tissues

The macroscopic examination revealed no major pancreatic lesions across all experimental groups. However, histopathological evaluation demonstrated substantial microscopic alterations in the LPS group. Specifically, marked degenerative and necrotic changes were detected in the β-cells of the islets of Langerhans, accompanied by intense hyperemia, interstitial edema, infiltration of inflammatory cells, and widespread parenchymal degeneration.

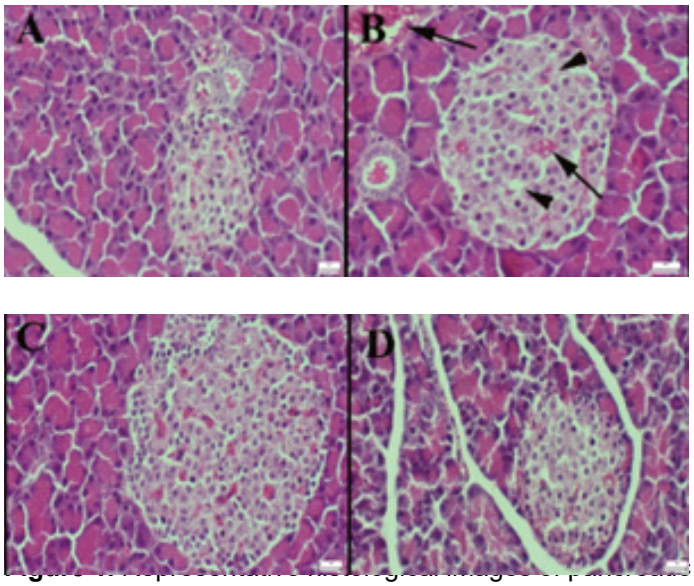

Figure 1. Representative histological images of pancreatic tissue from each experimental group. (A) Control group: Normal pancreatic architecture with well-preserved islets of Langerhans. (B) LPS group: Marked hyperemia (arrows) and degenerative cells (arrow heads) in the Langerhans islet. (C) LPS+AMA group: Noticeable reduction in pathological alterations. (D) AMA group: Pancreatic morphology appears normal, closely resembling that of the control group. HE, scale bar = 20 μm.

In contrast, the LPS+AMA group exhibited a remarkable attenuation of these pathological alterations. The architectural integrity of the pancreatic tissue, including the islets of Langerhans, was largely preserved in the LPS+AMA group compared to the untreated LPS group (Figure 1, 2).

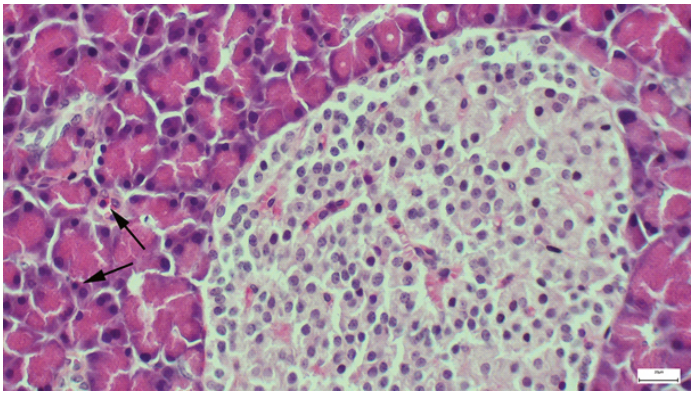

Figure 2. Histological section of pancreatic tissue in the LPS group demonstrating marked infiltration of inflammatory cells (arrows). HE, scale bar = 20 μm.

The LPS group demonstrated substantially higher hyperemia scores than the control group according to quantitative histopathological scoring ($p < 0.001$) which indicated vascular congestion. The LPS+AMA group showed a significant decrease in hyperemia scores ($p < 0.001$) which indicated AMA's protective effects on blood vessels (Figure 3). The LPS group displayed substantial edema scores above the control group ($p < 0.001$) yet the LPS+AMA group showed significant improvement ($p < 0.01$). The LPS group displayed higher inflammatory cell infiltration scores ($p < 0.001$) but AMA treatment reduced these scores ($p < 0.01$) which demonstrated its anti-inflammatory properties (Figure 3).

The degeneration scores demonstrated identical patterns where the LPS group displayed major structural damage ($p < 0.001$) but the LPS+AMA group obtained significantly lower scores ($p < 0.001$) which indicated successful histoprotective effects (Figure 3).

The histopathological results demonstrate that AMA effectively reduces the pancreatic damage caused by LPS particularly to islet β-cells.

Immunohistochemical Findings

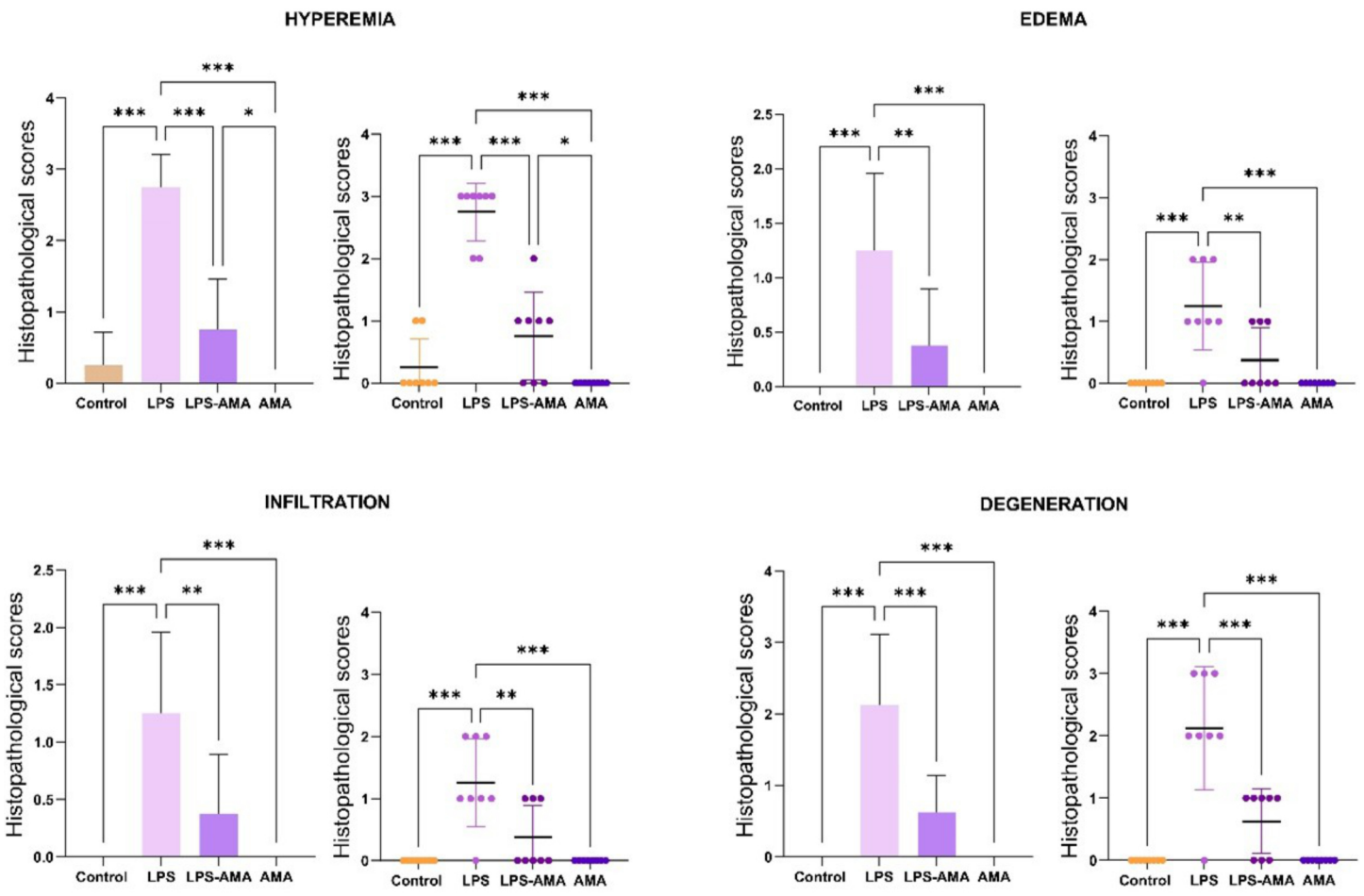

Figure 3. Histopathological evaluations of pancreatic tissue across all experimental groups.
Hyperemia, edema, inflammatory cell infiltration, and degeneration were scored semi-quantitatively using standard histological criteria. Each parameter was assessed using hematoxylin–eosin-stained sections. Bar graphs (left column) represent group mean ± SD values; dot plots (right column) show individual data points. Significantly increased histopathological scores were observed in the LPS group compared to control, indicating substantial pancreatic injury. Amantadine (AMA) treatment significantly reduced LPS-induced histological damage in all parameters. *p <0.05, **p <0.01, ***p < 0.001.

IFN-β immunostaining findings

Immunohistochemical staining revealed minimal IFN-β immunoreactivity in pancreatic sections from the control and AMA-alone groups. In contrast, the LPS group exhibited marked overexpression of IFN-β, particularly in the β-cells of the islets of Langerhans. The cytoplasm and perinuclear regions showed strong staining patterns which indicated a strong inflammatory reaction to endotoxin exposure.

Quantitative immunohistochemical scoring demonstrated a statistically significant increase in IFN-β expression in the LPS group compared to the control ($p < 0.001$). Treatment with AMA significantly reduced IFN-β levels in the LPS+AMA group compared to the LPS group ($p < 0.001$), though expression remained higher than in the control and AMA-alone groups ($p < 0.01$). These findings suggest that amantadine exerts a potent anti-inflammatory effect by downregulating type I interferon signaling in inflamed pancreatic tissue (Figure 4).

IL-1β immunostaining findings

IL-1β immunoreactivity was markedly increased in pancreatic tissue from the LPS group, particularly within the islets of Langerhans. The β-cells displayed intense cytoplasmic staining that indicated a strong pro-inflammatory reaction to endotoxemia. The IL-1β expression remained low in both control and AMA-alone groups.

Quantitative scoring of IL-1β immunostaining revealed a significant increase in the LPS group compared

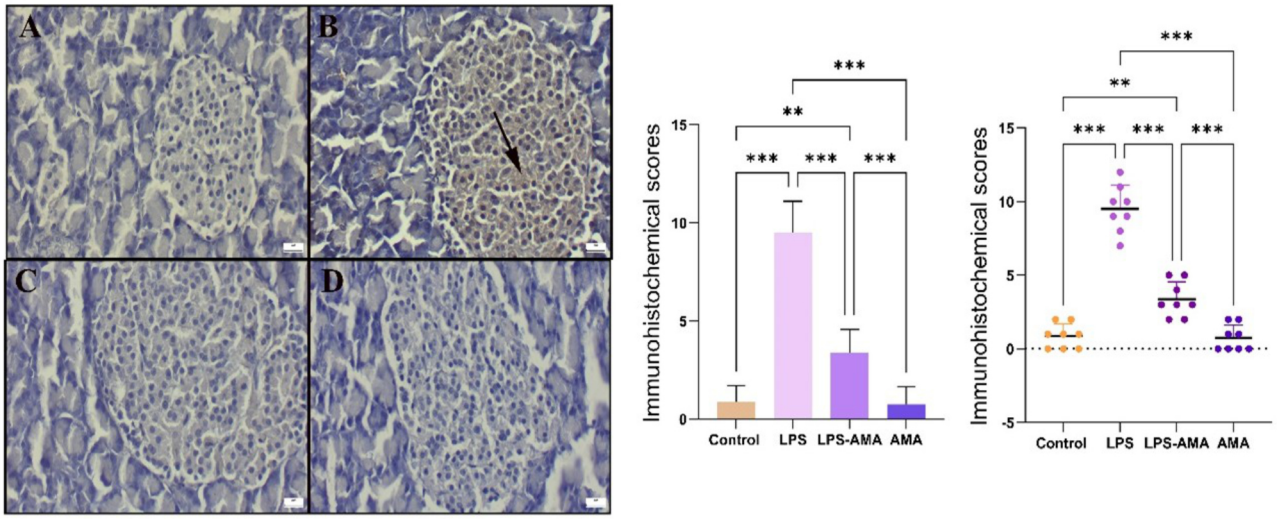

Figure 4. Representative immunohistochemical staining of IFN-β in the islets of Langerhans across experimental groups. (A) Control group: Negative immunoreaction, indicating preserved islet morphology and absence of inflammation. (B) LPS group: Increased immunoreactivity (arrow), reflecting significant inflammatory activation and cellular damage. (C) LPS+AMA group: Noticeable reduction in expression, suggesting the anti-inflammatory effect of AMA treatment. (D) AMA group: Negative to mild expression, similar to the control group, indicating no adverse effect on pancreatic tissue. Streptavidin biotin peroxidase method, Scale bars = 20 µm. Bar graph (left) and dot plot (right) represent immunohistochemical scores (mean ± SD and individual data points). **p < 0.01, ***p < 0.001.

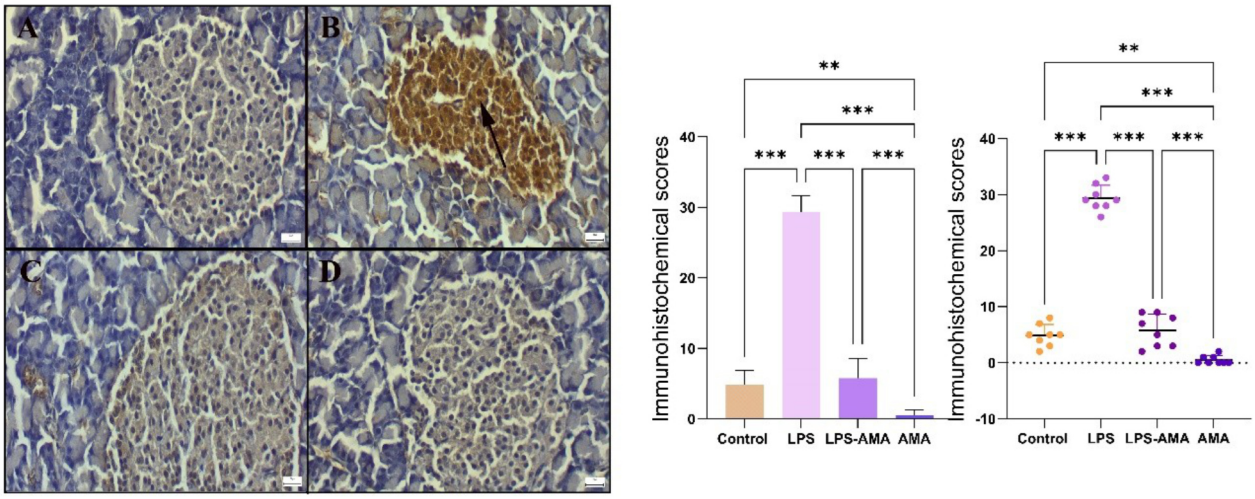

Figure 5. Immunohistochemical staining of IL-1β in the islets of Langerhans across experimental groups. (A) No expression observed in the Control group. (B) Strong positive immunoreactivity indicated by arrows in the LPS group. (C) Significant reduction in expression in the LPS+AMA group. (D) No expression detected in the AMA group. Staining was performed using the streptavidin-biotin peroxidase method. Scale bars = 20 µm. Graphical data are shown as mean ± SD (bar graph) with individual values represented in the dot plot. **p < 0.01, ***p < 0.001.

to the control (p <0.001). Treatment with AMA resulted in a statistically significant reduction in IL-1β expression in the LPS+AMA group (p <0.001 vs. LPS), though levels remained moderately elevated compared to controls (p <0.01). The results confirm that amantadine exhibits anti-inflammatory effects, mitigating pancreatic damage induced by cytokines (Figure 5).

IL-1β immunostaining findings

IL-1β immunoreactivity was markedly increased in pancreatic tissue from the LPS group, particularly within the islets of Langerhans. The β-cells displayed intense cytoplasmic staining that indicated a strong pro-inflammatory reaction to endotoxemia. The IL-1β

expression remained low in both control and AMA-alone groups.

Quantitative scoring of IL-1β immunostaining revealed a significant increase in the LPS group compared to the control ($p < 0.001$). Treatment with AMA resulted in a statistically significant reduction in IL-1β expression in the LPS+AMA group ($p < 0.001$ vs. LPS), though levels remained moderately elevated compared to controls ($p < 0.01$). The results confirm that amantadine exhibits anti-inflammatory effects, mitigating pancreatic damage induced by cytokines (Figure 5).

DISCUSSION

This study demonstrated that AMA administration significantly attenuates pancreatic tissue injury induced by LPS in rats, as evidenced by improvements in histopathological parameters and reductions in the expression of key inflammatory cytokines, including IFN-β, IL-1β, and TNF-α. The results indicate that amantadine protects pancreatic islets from damage through its ability to inhibit inflammatory pathways activated by LPS exposure.

LPS is a potent endotoxin known to induce a systemic inflammatory response through TLR4 activation, leading to the downstream release of pro-inflammatory mediators via NF-κB signaling (He et al., 2018). The inflammatory environment leads to tissue damage across various organs with vulnerability observed in the pancreas because of its minimal antioxidative defenses and extensive microvascular network (Dludla et al., 2023). In our study, LPS administration caused marked edema, hyperemia, inflammatory infiltration, and β-cell degeneration, consistent with previous models of endotoxemia-induced pancreatitis (Chen et al., 2025).

The overexpression of TNF-α and IL-1β observed in the LPS group reflects a classical M1 macrophage-dominated response, known to impair pancreatic endocrine function (Cnop et al., 2005). IL-1β, in particular, has been shown to promote nitric oxide production and reactive oxygen species generation, further exacerbating β-cell apoptosis (Eizirik et al., 2020). Our findings support this mechanism, with intense IL-1β and TNF-α immunoreactivity in the islets correlating with histological damage.

The research showed that AMA treatment reduced the expression of these cytokines which is consistent with previous studies that demonstrated its anti-inflammatory effects extend beyond the central nervous system. In experimental models of neuroinflammation, amantadine has been shown to reduce TNF-α and IL-1β levels via inhibition of microglial activation and NF-κB signaling (Jiménez-Jiménez et al., 2020). This supports our findings that amantadine may suppress similar pathways in peripheral tissues, such as the pancreas.

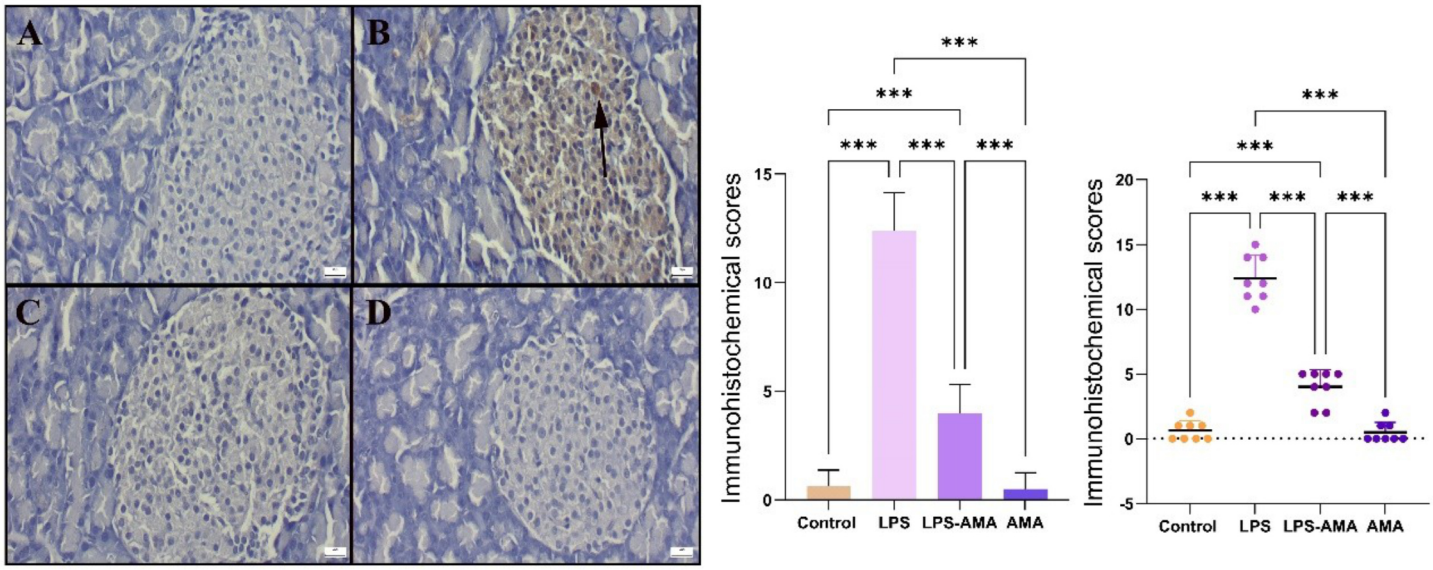

Figure 6. Representative immunoexpression of TNF-α in the islets of Langerhans between the groups. (A) No expression in control group (B) Increased expression in LPS group. (C) Decreased expression in LPS+AMA group. (D) Negative expression in AMA group. Streptavidin biotin peroxidase method, Scale bars = 20 μm. Bar and dot plots illustrate the immunohistochemical scores (mean ± SD and individual values). ***$p < 0.001$.

The observed decrease in IFN-β expression following amantadine treatment is particularly notable. IFN-β, a type I interferon, is upregulated during LPS challenge and contributes to both antiviral defense and tissue inflammation. Its sustained expression can lead to tissue injury and exacerbate apoptosis (McNab et al., 2015). In the pancreas, IFN-β signaling has been associated with β-cell dysfunction and insulitis (Lombardi and Tomer, 2017). Our study is among the first to show that AMA reduces IFN-β immunoreactivity in a peripheral organ under endotoxemic conditions.

The histopathological scores improved because AMA demonstrated cellular protective effects in addition to its cytokine suppression properties. This may involve modulation of oxidative stress, as shown in CNS models where amantadine reduced oxidative injury markers (Xing et al., 2018). Although oxidative stress markers were not evaluated in the present study, the reduced tissue damage and cytokine load indirectly suggest a possible antioxidative contribution.

The amantadine dosage and administration schedule in this research followed established protocols which showed protective impacts in ischemia-reperfusion and sepsis models (Sahin et al., 2023). Importantly, the lack of histopathological changes in the amantadine-alone group confirms the drug's safety profile at this dose and duration in pancreatic tissue.

The islets of Langerhans are uniquely sensitive to immune-mediated injury due to their limited regenerative capacity and proximity to perivascular immune cells. Long-term exposure to inflammatory mediators can result in long-term functional impairment which may contribute to post-septic metabolic dysregulation (Eizirik et al., 2020). Our findings that amantadine preserved islet architecture suggest that it could be a therapeutic agent in sepsis-associated endocrine dysfunction.

The current translational researches focuses on drug repurposing as an effective method to treat organ damage in sepsis patients. Amantadine's existing approval for neurological disorders and influenza, along with its well-characterized pharmacokinetics, makes it a strong candidate for further investigation in systemic inflammation (Kingsmore et al., 2020). The research contributes to the expanding body of evidence demonstrating AMA's multifunctional effects.

Nonetheless, the study has some limitations. We did not evaluate downstream intracellular signaling molecules such as NF-κB, STAT1, or NLRP3, which could provide mechanistic insight into amantadine's mode of action. The study did not include long-term pancreatic function assessments such as insulin level measurements which should be evaluated in future research.

In conclusion, our findings indicate that amantadine exerts protective effects in LPS-induced pancreatic injury by minimizing tissue damage and decreasing inflammatory cytokines in the islets of Langerhans. These results suggest a novel potential application for amantadine in preventing pancreatic dysfunction during sepsis.

CONCLUSION

In summary, this study demonstrates that amantadine significantly ameliorates LPS-induced pancreatic injury by reducing histopathological damage and suppressing the immunoexpression of key inflammatory cytokines such as IFN-β, IL-1β, and TNF-α within the islets of Langerhans. The protective effects of amantadine may stem from its ability to inhibit endotoxin-triggered cytokine signaling cascades and maintain islet architectural integrity. The research results demonstrate amantadine functions as an extra-neural therapeutic agent for treating sepsis-related pancreatic damage while showing promise for treating systemic inflammatory diseases.

ETHICAL APPROVAL

The Süleyman Demirel University Local Animal Ethics Committee examined and authorized every technique carried out on the rats (Approval no: 555). The Animal Research: Reporting of In Vivo Experiments (ARRIVE) 2.0 criteria were followed for conducting the study.

AUTHOR CONTRIBUTIONS

Idea, concept and design: ŞT, HA, ÖÖ, PK
Data collection and analysis: ŞT, HA, MÖ, ÖÖ, SG
Drafting of the manuscript: ŞT, HA, ÖÖ
Critical review: ŞT, HA, MÖ, ÖÖ, SG

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare in association with this study

RESEARCH FUNDING

This study was funded by Süleyman Demirel University's Scientific Research Projects Unit under project number TSG-2024-9515.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER-REVIEW

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