ORIGINAL RESEARCH

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Fluvoxamine Administration Attenuates Lipopolysaccharide-Induced Pancreatic Damage

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Abstract

Objective
Certain types of bacteria contain lipopolysaccharide (LPS), which can cause widespread inflammation in the body, including the pancreas. Fluvoxamine (FLV), a selective serotonin reuptake inhibitor (SSRI) commonly prescribed for psychiatric disorders, has been shown to possess anti-inflammatory properties and may be beneficial in conditions involving tissue damage and inflammation. This study aims to evaluate the potential protective effects of FLV against experimentally induced pancreatic disease in rats using LPS.

Material and Method
In this experiment, a total of 32 Wistar albino male rats were randomly divided into four groups: control, LPS (5 mg/kg, intraperitoneally (i.p.)), LPS + FLV (50 mg/kg FLV, i.p.) and FLV. The rats were euthanatized 6 hours after the administration of LPS, and serum and pancreas tissue samples were collected during the necropsy for biochemical, histopathological, and immunohistochemical evaluations.

Results
According to the study findings, LPS lowered blood glucose levels. Histological examination showed that LPS caused edema, mild infiltration of inflammatory cells, increased vacuolization in the cells of the Langerhans islet, and severe hyperemia. Immunohistochemical investigations revealed a reduction in the expression of insulin and amylin. The biochemical, histopathological, and immunohistochemical outcomes were improved by FLV.

Conclusion
The results of this experimental rat model study indicated that LPS causes damage to the endocrine pancreas. However, FLV demonstrated significant ameliorative effects on the pancreas in rats with LPS-induced pancreatitis.

Keywords: Biochemistry, IHC, LPS, pancreas, pathology

Introduction

Lipopolysaccharide (LPS) is a compound found in bacterial cell membranes, commonly known as an endotoxin. These endotoxins are potentially harmful molecules that can trigger inflammation and immune responses in the body. LPS, specifically found in the cell walls of bacteria in the intestinal microbiota,

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can be released through various processes in the intestines, allowing it to enter the circulatory system and, potentially cause damage to various organs (1-8). The pancreas is an organ that produces digestive enzymes and hormones (9). When LPS enters the circulatory system, the systemic inflammation it causes can lead to pathological changes in the pancreas resulting in conditions such as pancreatitis (7,8). The hormones insulin, glucagon, and amylin which are secreted by the pancreas, provide critical information about pancreatic damage (10).

Fluvoxamine (FLV) is an antidepressant belonging to the selective serotonin reuptake inhibitor (SSRI) class. This medication influences neurotransmission in the central nervous system by regulating serotonin levels and, is commonly used to treat psychiatric conditions such as major depressive disorder, obsessive-compulsive disorder (OCD), panic disorder, and social anxiety disorder (11).

FLV works by preventing the reuptake of serotonin from nerve terminals, thereby increasing serotonin levels between nerve cells. This action allows the neurotransmitter to remain in synapses for a longer duration, communication between nerve cells. Serotonin is a crucial neurotransmitter that regulates mood, sleep, appetite, and overall behavior (11,12).

Recent research has indicated that FLV may possess anti-inflammatory and immunomodulatory properties potential and benefits in treating conditions associated with inflammatory processes (13).

LPS exerts its harmful effects on the pancreas primarily by inducing inflammation (7,8,14). This experimental rat model study aims to investigate the cellular-level effects of FLV on LPS-induced pancreatic pathology.

Material and Method

Animals and Study Design

All experiments conducted for this study adhered to the ARRIVE (Animal Research Reporting in Live Experiments) 2.0 guidelines for animal research.

Thirty-two Wistar Albino male rats were obtained from the Experimental Animals Laboratory of SDU and randomly divided into four groups.

Group 1 (Control, n=8): 0.5-1 ml physiological saline (PS) was administered intraperitoneally (i.p.) to the left inguinal regions of the rats, followed by 0.5 ml of PS to the right inguinal regions 30 minutes later.

Group 2 (LPS, n=8): After the i.p. administration of 0.5-1 ml PS to the left inguinal regions, 5 mg/kg of LPS in a volume of 0.5 ml was administered i.p. to the right inguinal regions 30 minutes later. The LPS, in solid form, was dissolved in PS (15).

Group 3 (LPS+ FLV, n=8): 50 mg/kg of FLV (16) dissolved in PS in a volume of 0.5-1 ml, was administered i.p. to the left inguinal regions. Thirty minutes later, 5 mg/kg of LPS in a volume of 0.5 ml was administered i.p. to the right inguinal regions.

Group 4 (FLV, n=8): 50 mg/kg FLV, dissolved in PS in a volume of 0.5-1 ml, was administered i.p. to the left inguinal regions. Thirty minutes later, 0.5 ml PS was administered i.p. to the right inguinal regions.

The experimental animals were sacrificed with 80–100 mg/kg of ketamine (Ketasol, Richter Pharma AG, Austria) and 8–10 mg/kg of xylazine (Xylasinbio%2, Bioveta, Czech Republic) six hours following the last drug administration. Serum glucose levels are measured using blood samples. All rats had their pancreatic tissue samples carefully removed, and they were fixed in a 10% formaldehyde solution for immunohistochemical and histopathological analyses.

Biochemical Analyses

Serum glucose levels were determined using a spectrophotometric method with a commercial kit (glucose GOD FS-1 2500 99 10 923, DiaSys, Holzheim, Germany) compatible with an autoanalyzer (Beckman Coulter AU5800, USA).

Histopathological Assessment

During necropsy, pancreatic tissue samples were collected and fixed in a 10% buffered formaldehyde solution. After two days of fixation, the tissues underwent standard processing with an automated tissue processing device. They were then embedded in paraffin wax, and sliced into 5µm sections using a rotary microtome (Leica Microsystems, Wetzlar, Germany). The sections were stained with hematoxylin-eosin (HE) and examined under a light microscope in a blinded manner. The pancreas was semi quantitatively scored for hyperemia, hemorrhage, edema, inflammation, degeneration, and necrosis on a scale of 0-3.

Immunohistochemical Analysis

Three consecutive sections of pancreatic tissue samples were immunostained for insulin (Anti-Insulin antibody [EPR17359] (ab181547)), amylin (Anti-Amylin/DAP antibody [EPR22556-138] (ab254259)), and glucagon (Anti-Glucagon antibody [EP3070] (ab92517)) following histopathological examination

to assess the expression of these markers. Immunostaining was performed using the streptavidin-biotin peroxidase technique according to the manufacturer's instructions. The secondary antibody used was the Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab64264), with diaminobenzidine (DAB) serving as the chromogen. Both primary and secondary antibodies were supplied by Abcam (Cambridge, UK). For negative controls, antibody dilution solutions were used in place of the primary antiserum step.

A specialized histopathologist from a different university, who was unaware of group assignments, evaluated each examination. Under X40 objective magnification, the percentage of cells positively immunostained for each marker was calculated in 10 different fields for each section across all groups. Image analysis was done using ImageJ software (version 1.48, National Institutes of Health, Bethesda MD). Microphotographs were generated using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical Analysis

The data obtained were subjected to statistical analysis using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Group comparisons were conducted using ANOVA, and variable assessments were performed with the Tukey test. Statistical significance was set at $p < 0.05$.

Results

When blood glucose levels were examined across the groups, it was observed that LPS administration significantly reduced blood glucose levels. Conversely, FLV applications were found to be effective in normalizing the levels. Blood glucose levels for each group are presented in Figure 1.

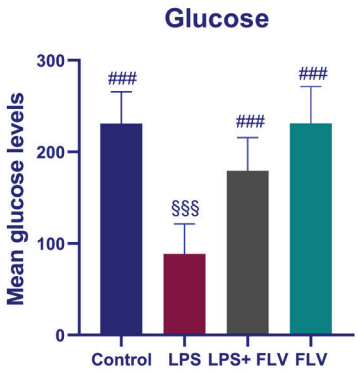
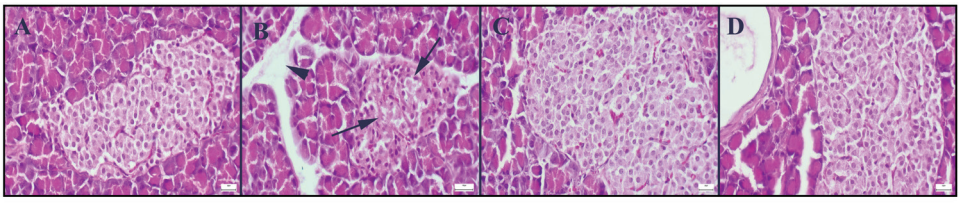
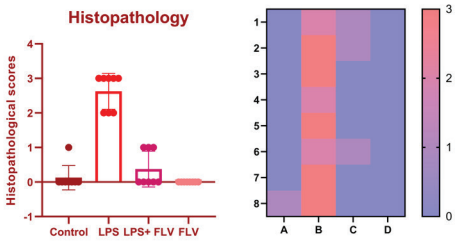


Figure 1:
Blood glucose levels (mg/dl) between the groups, differences between groups with different superscripts are statistically significant, p<0.001.(a) Ca, (b) PTH, (c) age, and (d) NLR levels for predicting postoperative hypocalcemia.



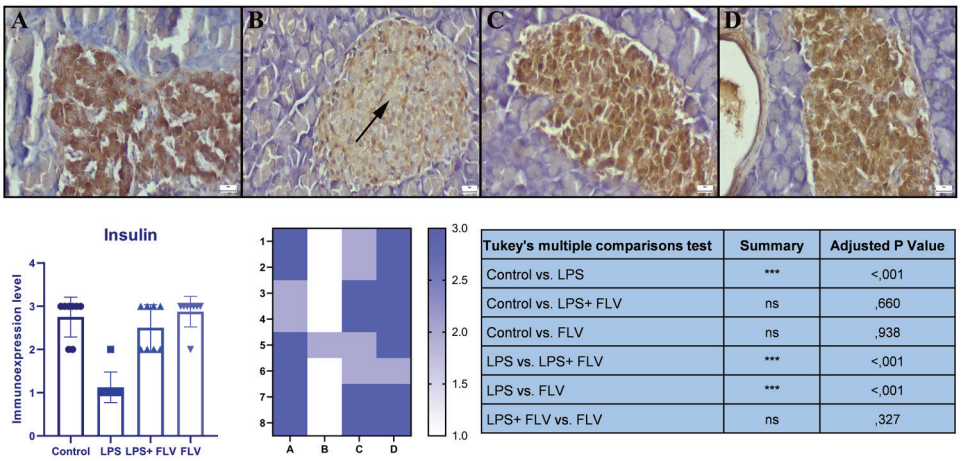


Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. LPS	***	<,001
Control vs. LPS+ FLV	ns	,613
Control vs. FLV	ns	,926
LPS vs. LPS+ FLV	***	<,001
LPS vs. FLV	***	<,001
LPS+ FLV vs. FLV	ns	,274

Figure 2:
Representative histopathological images of the pancreas between the groups and statistical analysis results of histopathological scores. (A) Normal pancreatic histoarchitecture in a rat from the control group. (B) Marked edema (arrowhead) and numerous necrotic cells (arrows) in pancreatic Langerhans islets a rat belonging LPS group. (C) Almost normal histological appearance in pancreatic Langerhans islet cells in a rat form LPS+FLV group. (D) Normal pancreatic histology in a rat in FLV group, HE, Scale bars= 20µm.

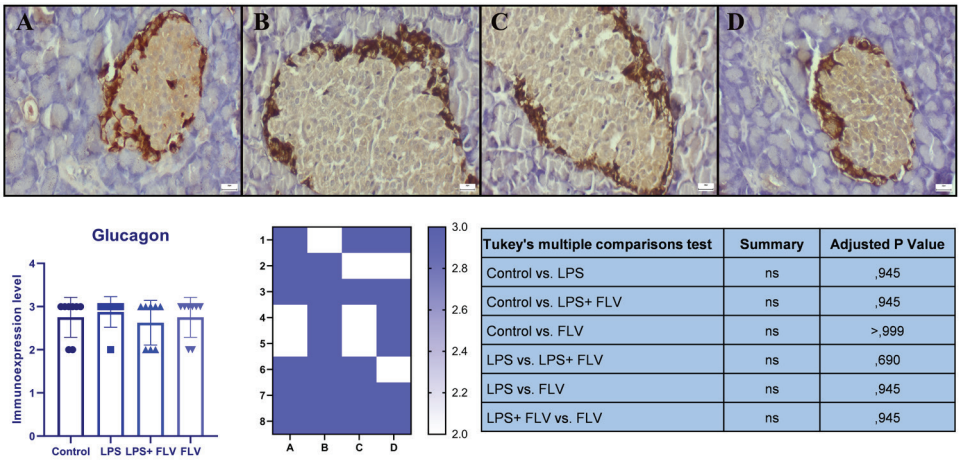
The pancreatic tissue in the control rats appeared normal. Histopathological analysis of the pancreas in the LPS group showed markedly elevated levels of endocrine cell vacuolization, edema, and moderate infiltrations of inflammatory cells, primarily neutrophils. Additionally, single-cell necrosis in the Langerhans islets was also noticed in the LPS group. The administration of FLV resulted in an improvement in these pathological findings. Microscopic examination of the pancreas in the FLV group showed no abnormalities (Fig. 2).

During the immunohistochemical analysis, glucagon-immunopositive cells were found at the periphery of the Langerhans islet, whereas amylin- and insulin-immunopositive cells were observed in the center region. Each of the three markers was located in the cytoplasm. The analysis indicated that LPS administration markedly decreased the expression of insulin and amylin, while there was no significant change in glucagon level. Additionally, in the LPS group, both insulin and amylin expressions showed a significant decrease compared to the control group



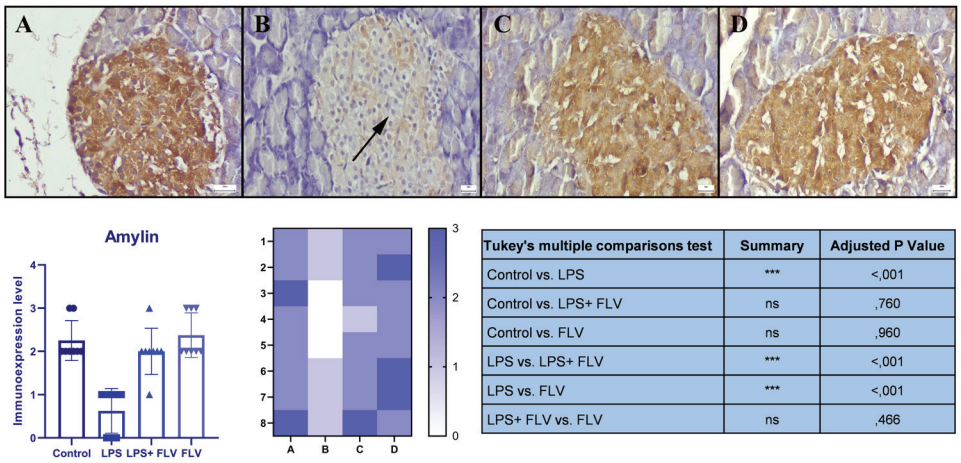
Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. LPS	***	<,001
Control vs. LPS+ FLV	ns	,660
Control vs. FLV	ns	,938
LPS vs. LPS+ FLV	***	<,001
LPS vs. FLV	***	<,001
LPS+ FLV vs. FLV	ns	,327

Figure 3:
Insulin immunoexpressions in the endocrine islet of the pancreas among the groups and statistical analysis results of insulin IHC scores. (A) Significant expressions in the control group. (B) Decreased 7 expression (arrow) in the LPS group. (C) Markedly increased in expressions in the LPS+FLV group. (D) Normal significant expressions in all markers in the FLV group. Streptavidin biotin peroxidase method, scale bars=20µm.



Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. LPS	ns	,945
Control vs. LPS+ FLV	ns	,945
Control vs. FLV	ns	>,999
LPS vs. LPS+ FLV	ns	,690
LPS vs. FLV	ns	,945
LPS+ FLV vs. FLV	ns	,945

Figure 4:
Glucagon immunoexpressions in the endocrine islet of the pancreas among the groups and statistical analysis results of glucagon IHC scores. Similar expressions at the peripheral area of the Langerhans islet in the (A) control group, (B) LPS group, (C) LPS+FLV group, and (D) FLV group. Streptavidin biotin peroxidase method, scale bars=20µm.



Tukey's multiple comparisons test	Summary	Adjusted P Value
Control vs. LPS	***	<,001
Control vs. LPS+ FLV	ns	,760
Control vs. FLV	ns	,960
LPS vs. LPS+ FLV	***	<,001
LPS vs. FLV	***	<,001
LPS+ FLV vs. FLV	ns	,466

Figure 5:
Amylin immunoexpressions in Langerhans islet of the pancreas in the groups and statistical analysis results of amylin IHC scores. (A) Marked expressions in the control group. (B) Decreased expression (arrow) in the LPS group. (C) Increased in expressions in the LPS+FLV group. (D) Marked expressions in the FLV group. Streptavidin biotin peroxidase method, scale bars=20µm.

($p < 0.001$ for both), but the decrease in glucagon expression was not statistically significant ($p > 0.05$). Following treatment with FLV in the LPS-FLV group, Amylin and insulin levels returned to normal. Likewise, insulin and amylin levels were significantly higher in the FLV group compared to the LPS group ($p < 0.001$). The glucagon expressions in the FLV-treated groups did not differ significantly from the other groups ($p > 0.05$ for all) (Fig. 3-5).

Discussion

In this study, the effects of FLV, an antidepressant belonging to the selective serotonin reuptake inhibitors (SSRI) class, were investigated for the first time against LPS-induced pancreatic damage. The impact of LPS on the pancreas was assessed, and the therapeutic effects of FLV were identified.

LPS is a molecule found in bacterial cell walls that can stimulate the immune system and initiate inflammatory responses. Excessive activation of LPS can contribute to inflammatory diseases and overstimulation of the immune system, leading to harmful effects on various tissues. The pancreas is particularly sensitive to LPS and is severely affected by its actions. The Langerhans islets are the most impacted part of the pancreas (7,8,17).

Blood glucose levels and overall metabolism are greatly influenced by the endocrine activity of the pancreatic islets, which produce glucagon, insulin, proinsulin, somatostatin, pancreatic polypeptide, amylin and C-peptide. Glucagon elevates blood glucose levels while insulin reduces them. Insulin secreted by the pancreas, is essential for the metabolism of proteins, fats, and carbohydrates. Since glucose is a key energy source for immune cells, there is a connection between glucose metabolism and immunological function (18,19).

Elevated blood glucose levels have been linked to immune cell malfunction and apoptosis through oxidative stress and inflammation. On the other hand, low blood glucose levels can weaken the immune system and make a person more vulnerable to diseases. According to the study, exposure to LPS raises blood glucose levels and decreases pancreatic insulin expression. It is thought that LPS damages the cells that secrete insulin, preventing its production (8). However, the decrease in blood glucose that occurs when exposed to LPS is complicated. As a defensive mechanism, immune cells may enhance the uptake of glucose, and hepatic gluconeogenesis inhibition may also impact glucose homeostasis. Because LPS inhibits hepatic glucose synthesis, severe endotoxic shock might impair the body's ability to maintain glucose homeostasis, resulting in hypoglycemia (8, 20–22). In this study, serum glucose levels in this study dropped during necropsy and six hours after LPS injection. This decrease is thought to be caused by hyperinsulinemia that occurs just after LPS delivery, which stresses the beta cells in the pancreas responsible for producing insulin. Long-term hyperinsulinemia may be linked to pancreatic alterations and malfunction of the beta cells.

The pancreas can be damaged due to various reasons, and one significant indicator of damage to the Langerhans is the impairment of insulin expression. Particularly in conditions that lead to inflammation, degeneration, and necrosis, the synthesis of insulin tends to decrease. The endocrine part of the pancreas, consisting of more delicate cells compared to the exocrine part, is particularly susceptible to damage. Therefore, damage is more likely to occur in the endocrine cells of the pancreas, with cells responsible for insulin synthesis being the most vulnerable among the endocrine cells (7-9). Similar findings were observed in this study, where LPS markedly decreased in insulin expressions in the Langerhans islet.

Amylin is a hormone secreted by the pancreas that typically works in conjunction with insulin to help regulate blood glucose levels. Pancreatic damage, especially in conditions affecting the Langerhans islets, can impact amylin levels. Cellular damage in the pancreas can lead to irregularities in amylin production. This condition can result from diseases such as pancreatitis, pancreatic tumours, or other inflammatory conditions. Pancreatic damage can hinder the normal secretion of amylin and other digestive enzymes, leading to disruptions in the digestive process. Therefore, conditions associated with pancreatic damage can cause changes in amylin levels, thereby affecting the regulation of blood glucose (23,24). It has been reported that amylin expression decreases in pancreatic injuries (7,8). The findings of this study demonstrate parallelism with the prominent findings in the literature regarding the effects of LPS on amylin expression.

Glucagon is a hormone released from the pancreas and typically has the opposite effect of insulin. In situations where insulin levels are low, glucagon is released, promoting the conversion of glycogen to glucose in the liver and increasing blood glucose levels. Pancreatic damage, especially in cases where Langerhans islets are affected, can influence glucagon levels. Cellular damage in the pancreas can lead to irregularities in glucagon production. This condition may result from diseases such as pancreatitis, pancreatic tumours, or other inflammatory conditions. Pancreatic damage can disrupt the normal regulation of glucagon, leading to disturbances in blood glucose control. Therefore, conditions associated with pancreatic damage can cause changes in glucagon levels and, consequently, difficulties in regulating blood glucose (25). It has been reported that glucagon expression are less affected or not affected at all in pancreatic damage compared to insulin (7,8). In this study, a slight increase was observed in the cells synthesizing glucagon, but this increase was statistically insignificant. This result further supports the idea that the cells producing glucagon are among those least affected by LPS

LPS, a molecule found in bacterial cell walls, can stimulate the immune system and initiate inflammatory responses. The pancreas is a critical organ in the body, playing a pivotal role in digestion and energy metabolism. Excessive activation of LPS can contribute to the occurrence of inflammatory diseases and other conditions affecting the immune system (7,8). The pancreas is particularly sensitive to LPS and is one of the organs significantly affected by its impact. In cases of pancreatic damage, structures within the pancreas, such as the Langerhans islets, may be influenced by the effects of LPS. This situation can arise as a result of inflammatory conditions like pancreatitis or bacterial infections. Pancreatic damage may be associated with increased inflammatory responses in pancreatic tissue induced by LPS (7,8,26).

The findings of the present study demonstrate that LPS causes damage in the pancreas, particularly in the cells of Langerhans islets, and FLV is effective in preventing this damage at the cellular level. It is believed to exert its effect through its anti-inflammatory properties. More research is required on this topic.

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Conflict of Interest Statement
There is no potential conflict of interest.

Ethical Approval
The experimental protocol and ethical requirements of the study were approved by the Suleyman Demirel University Animal Experimentation Local Ethics Committee, with approval 01.03.2024 date number 265.

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Availability of Data and Materials
Data is available on request from the authors.

Authors Contributions
ŞT: Conceptualization; Data curation; Formal analysis; Methodology; Validation; Visualization; Writing-original draft.

ÖÖ: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing-review & editing.

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