# Investigation of Antimicrobial Effect of Fluoxetine in Experimental Rat Sepsis Model

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**Abstract**

Background and Purpose: Some studies showed that fluoxetine has some promising properties in the treatment of specific infections; however, its effects have not been studied in the sepsis model. This research aims to investigate the effect of fluoxetine on the inflammatory process in a sepsis model in rats and to investigate its efficacy in modifying the antibiotic effect of imipenem. Experimental Approach: 40 rats were equally divided into five groups. The first group is as a negative control, group 2 is a positive control, group 3 treated with fluoxetine 5mg/kg, group 4 treated with Imipenem antibiotic 60mg/kg, and group 5 treated with fluoxetine combined with imipenem for 72 hours. The expression level of serum and tissue HsCRP, pro-calcitonin (PCT), lactate, myeloperoxidase activity (MPO), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNFα), and monocyte chemoattractant protein-1 (MCP-1) was measured using ELISA. Oxidative stress markers were measured using photometric methods, total thiol (TT), native thiol (NT), total oxidant status (TOS), and total antioxidant status (TAS). Total tissue protein concentrations were measured by the Bradford method. Key Results: In fluoxetine, imipenem, and combined (fluoxetine + imipenem) groups, the IL-1β, IL-6, TNF-α, MPO, MCP-1, HsCRP, PCT, lactate, TOS, OSI, and disulfide levels were reduced ($p<0.05$). The antioxidant indicator (TT, NT, and TAS) levels significantly increased ($p<0.05$). Fluoxetine and imipenem combined therapy showed positive synergistic effects. Conclusion and Implications: This research shows that fluoxetine has an anti-inflammatory and antioxidant effect and its combined therapy with imipenem shows positive synergistic effects in the experimental sepsis model.

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Keywords: sepsis, inflammation, antidepressive, antibiotic, cytokines, imipenem, fluoxetine, rat

# 1. Introduction

According to the Centers for Disease Control and Prevention (CDC), more than 1.5 million of these infection cases turn into 'sepsis', which can be defined as a syndrome of physiologic, pathologic, and biochemical abnormality (Dantes & Epstein, 2018). Sepsis is a major public health problem comes with a startling economic burden accounting for more than $20 billion (5.2%) of total hospital costs in the US and £1.5-2.0 billion each year in the UK (Synger et al., 2016; Wentowski, Mewada, & Nielsen, 2019).

As there still is no gold-standard to diagnose sepsis, 3 international conferences arranged in 1991, 2001, and 2016 have attempted to clarify the definition of sepsis and revise the treatment with the new understandings of its pathophysiology. In The Third International Consensus Definitions for Sepsis and Septic Shock, sepsis re-defined as 'dysregulated or exaggerated host response to infection-causing mortal organ dysfunction' (Gül, Arslantaş, Cinel, & Kumar, 2017; Synger et al., 2016).

The activation of innate immune cells is the initiation of the host response to the pathogen, which is mainly composed of monocytes, macrophages, neutrophils, and natural killer cells. This can occur via the binding of pathogen-associated molecular patterns (PAMPs) such as bacterial endotoxins on these cells to specific pattern recognition receptors. On the other side, another interaction is damage-associated molecular patterns (DAMPs). DAMPs can be intracellular molecules released from dead or damaged host cells, such as mitochondria and ATP. These bind to toll-like receptors (TLRs), which is a specific receptor on monocytes and macrophages. These activate the intracellular signal transduction pathway that causes the transcription and release of proinflammatory cytokines TNF-α, IL-1β, and IL-6. TNF-α has an important role in sepsis (Gyawali, Ramakrishna, & Dhamoon, 2019). The circulating levels of TNF-α are higher in septic patients compared with critically ill non-septic patients (Johansen, 2015). Some of the pattern recognition receptors can aggregate into larger protein complexes (inflammatory masks). These are responsible for the production of cytokines IL-1β and IL-18 concerned with programmed cell death. Proinflammatory cytokines cause activation and proliferation of leukocytes. In addition, these cytokines cause the production of tissue factors, induction of hepatic acute phase reactants, and activation of the complement system. There is an overstatement of this pathway which results in collateral damage and the death of tissues in sepsis (Gyawali et al., 2019).

Sepsis can be caused by bacteria, viruses, fungi, parasites, as well as non-infectious events such as severe trauma or pancreatitis. Although no inductor can be shown in approximately half of the cases, the majority of this group responds to antibiotic therapy, suggesting that the inducting agent may also be bacterial in

these patients. The microorganism that enters the body can lead to a variety of presentations, from infection and bacteremia to sepsis, and septic shock leading to death (Bennett, Dolin, & Blaser, 2014).

While immune system cells are generally beneficial, an excessive and unregulated inflammatory response causes severe organ damage. During sepsis, organ dysfunction ensues from cytotoxic injury, tissue ischemia, and apoptosis (Arwyn-Jones & Brent, 2019). Conditions such as systemic hypotension, impaired perfusion of microcirculation lead to tissue toxicity and contribute to organ failure (Rossaint & Zarbock, 2015).

There is an association between inflammation and depression. One of the pathways is that inflammation and cytokines may alter serotonin metabolism (Dantzer, O'Connor, Lawson, & Kelley, 2011). Antidepressants may have anti-inflammatory properties, and from the opposite way, there is an ongoing debate about the efficacy of nonsteroid anti-inflammatory drugs (NSAIDs) in some types of depression (Eyre, Air, Proctor, Rositano, & Baune, 2015). In any case, it could be concluded that inflammatory processes play a significant role in depression.

Multiple organ dysfunction syndromes (MODS) is the dysfunction of organ function in patients with sepsis and is the most important cause of mortality (Fry, 2012). The risk of death for each organ failure increases by 15-20%. Insufficiency of the coagulation system, liver, gastrointestinal system, and kidneys are common problems in sepsis that increase mortality (Sungur, 2005).

In the liver, sepsis disrupts hepatic clearance. This results in impairment of the clearance of bacteria and their products entering the portal system from the gut. This may subsequently spill over into the systemic circulation (Arwyn-Jones & Brent, 2019). According to clinical and experimental data, liver dysfunction is noted to be an early symptom of sepsis (Yan, Li, & Li, 2014). Sepsis results can be divided into primary and secondary liver dysfunction. Primary liver dysfunction develops after septic shock and is associated with systemic microcirculation disorders. In addition, liver hypoperfusion develops in sepsis and causes primary liver damage along with endotoxemia. Secondary liver dysfunction is caused by the inflammatory response caused by endotoxin activating cytokines. Cytokines in the liver that cause neutrophil activation from Kupffer cells are secreted (Szabo, Romics Jr, & Frendl, 2002).

Acute kidney injury (AKI) is a common and severe consequence of sepsis. there is evidence that sepsis and septic shock are the most important causes of AKI in critical patients. These patients constitute more than 50% of AKI cases in intensive care units and cause very high mortality (Zarjou & Agarwal, 2011). Since sepsis is associated with normal or increased renal blood flow, acute kidney injury that develops as a result of damage caused by hypoperfusion and associated hypoxemia is not a sufficient explanation. However, there is a change in the distribution of this blood flow from the cortex to the medulla. Mechanisms may include direct renal vasoconstriction, the release of proinflammatory cytokines, and activation of neutrophils in the renal vasculature (Takasu et al., 2013).

Typical circulatory abnormalities in sepsis can suppress the normal barrier function of the intestine, resulting in the translocation of bacteria and endotoxins into the systemic circulation. This leads to an increase in septic response (Luce, 1987). This was supported by sepsis animal models and a prospective cohort study (Doig et al., 1998).

The key priority in the management of sepsis is early diagnosis and treatment. Treatment includes antibiotics, organ support, and source control (Goodwin et al., 2015). Antibiotics, when they are used in a wisely and timely manner, cater as a great tool in combatting the attacks of microorganisms. But antibiotics alone are not enhancements, which will improve the overall septic appearance. It's known that antibiotics trigger the release of bacterial cell wall components, which partake in the severe inflammation that leads to sepsis in the body. Besides, antibiotic resistance is an important issue and has directed researchers to several non-antibiotic products and drugs. Many non-antibiotic drugs (non-steroidal anti-inflammatory drugs (NSAIDs), mucolytic agents, calcium channel blockers, and proton pump inhibitors) have some influence on the physiology and the viability of microorganism.

Psychotropic drugs are used to treat depression and other mental illnesses. Antidepressants help in the

reduction in depressive symptoms by altering chemical imbalances of neurotransmitters located in vesicles found in nerve cells of the brain. When the hippocampal inflammation hypothesis was first put forward, researchers mainly focused on whether depression can be treated with non-steroidal anti-inflammatory drugs. When the other side of the coin started to be considered, the question arose whether antidepressants that work in the treatment of depression have a therapeutic effect on other systemic inflammation conditions.

The antimicrobial effects of chlorpromazine, a dopamine antagonist, were found in 1959, and then studies targeted serotonin. After an antipsychotic, antidepressants were also included in the category of antimicrobial effective non-antibiotic drugs. The new antidepressants sertraline, fluoxetine, and paroxetine, are known to act as efflux pump inhibitors in human cells. The influence of these non-antibiotic alternatives occur in several ways, namely through direct antimicrobial activity, modification of the antimicrobial activity of antibiotics by increasing the efficiency of an antibiotic when given together, and activity on the physiology and pathogenicity of microorganisms (Kalaycı, Demirci, & Sahin, 2014; Munoz-Bellido, Munoz-Criado, & Garcıa-Rodrıguez, 2000).

Fluoxetine is a widely known drug of choice for the treatment of depression; it is an antidepressant drug that belongs to the Selective Serotonin Reuptake Inhibitor (SSRI) family (Charles et al., 2017). Studies showed that fluoxetine increases the peripheral blood mononuclear cells and B lymphocytes. Moreover, fluoxetine inhibits lipopolysaccharide (LPS) induced production of tumor necrosis factor-alpha (TNF-α), interleukin- 6 (IL-6), and nitric oxide (NO) (Charles et al., 2017; Liu et al., 2011). In an *in-vitro* study, fluoxetine showed a significant antibacterial effect and potential antibiotic modulating activity against multiresistant bacteria. Fluoxetine, together with an antibiotic such as gentamicin and erythromycin, used against *P. aeruginosa* and *E. coli*showed synergistic effects. It demonstrates that fluoxetine can selectively modulate the activity of antibiotics for clinical use (de Sousa et al., 2018). Furthermore, it has anti-inflammatory effects in animal models of peripheral inflammation. The neuroprotective effect of fluoxetine was assessed in an experimental pneumococcal meningitis animal model (Liechti, Grandgirard, & Leib, 2015). Fluoxetine was found to suppress the number of inflammation-related cells and TNF-α release from monocytes in lipopolysaccharides (LPS)-induced septic shock and allergic asthma animal model (Roumestan et al., 2007). Duda & Kubera (2017) found the anti-inflammatory effect of fluoxetine in aged female mice was evidenced by 1) reduction of pro-inflammatory cytokine production in the spleen and hippocampus, 2) enhancement of anti-inflammatory IL-10 production in the spleen, and 3) inhibition of the proliferative activity of Con A-stimulated splenocytes (Duda et al., 2017).

# 2. Material and method

## Materials

Ninhydrin, acetic acid, aluminum chloride ($AlCl_3$), cadmium chloride hemi (pentahydrate), (+) quercetin, methanol, sodium hydroxide (NaOH), gallic acid, L-Leucine, 2,4,6-tripyridyl-S-triazine (TPTZ), sodium nitrite ($NaNO_2$), potassium persulphate ($K_2SO_4$), ferric chloride ($FeCl_3$), sodium chloride (NaCl), sodium carbonate ($Na_2CO_3$), ferrous ammonium sulfate, phosphoric acid ($H_3PO_4$), Coomassie Brilliant Blue, and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Methanol, ethanol, ethyl acetate, and orthophosphoric acid were purchased from Merck Chemical (Darmstadt, Germany). Imipenem was purchased from Merck, Sharp & Dohme (Madrid, Spain) and fluoxetine HCl was obtained as commercially available 20-mg capsules (Prozac; Lilly Co., Madrid, Spain).

## Ethical considerations

*Bezmialem Vakıf University Animal Experiments Local Ethics Committee has approved this research study by the code number (2019/121).*

## Protocols of Experimental sepsis model

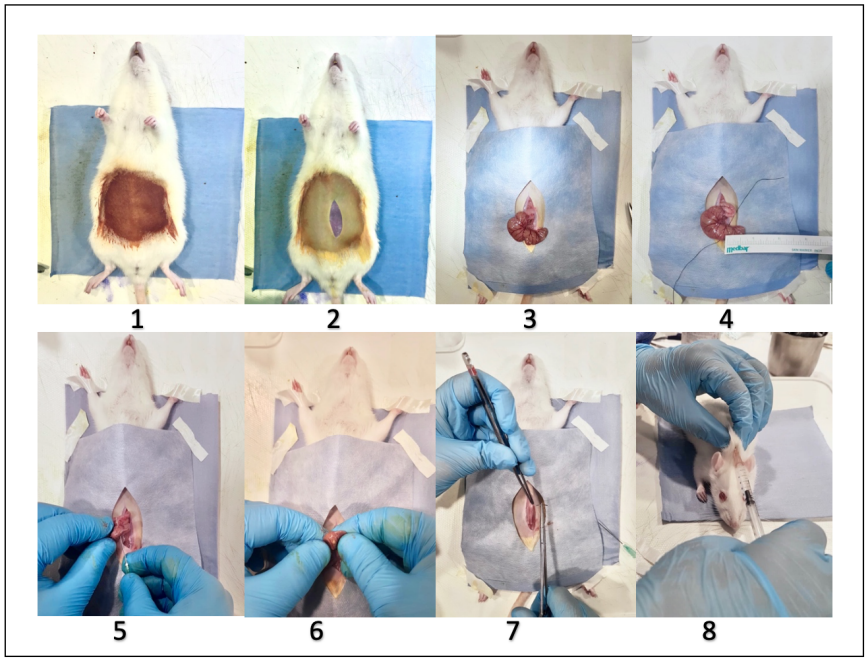
### 2.3.1 Experimental Graph

This study was conducted on 40 three-month-old female Wistar Hannover rats, weighing 230-250 grams purchased from the Bezmialem Vakıf University Experimental Animal units. The rats were housed in cages with a 12h light/dark cycle at room temperature. All the rats had free access to regular rodent diet food and tap water.

The rats were divided into five groups (n=8 per group):

- Group 1 (Negative Control): This group had undergone a surgical incision called "Sham Operation" to mimic the same after-surgical effects. The rats did not have any treatment, only were given a Saline solution (0.9 NaCl) every 12 hours through 72 hours.
- Group 2 CLP (Positive Control): This group had undergone a surgical procedure which is called the Cecal Ligation & Puncture (CL&P) to mimic sepsis syndrome. The rats did not have any treatment, only were given a Saline solution (0.9% NaCl) every 12 hours through 72 hours.
- Group 3 (CLP + Fluoxetine): The CL&P method applied, and rats received 5 mg/kg Fluoxetine (FLU) intraperitoneally every 12 hours through 72 hours (10 mg/kg/day).
- Group 4 (CLP + Imipenem): The CL&P method was applied, and rats received 60 mg/kg Imipenem (IMP) intraperitoneally every 12 hours through 72 hours (120 mg/kg/day).
- Group 5 (CLP + Fluoxetine + Imipenem): The CL&P method applied, and rats received 5mg/kg Fluoxetine (FLU) and 60 mg/kg Imipenem (IMP) intraperitoneally every 12 hours through 72 hours (10 mg/kg/day & 120 mg/kg/day).

Since the aim of our study was to see the antimicrobial effect of fluoxetine and its ability to modify the potency of antibiotics, the frequently used LPS sepsis model was not the right option for us. Therefore, the Cecal ligation and puncture (CLP) model was used. Small modifications were made to the method described by Rittirsch et al. (Rittirsch, Huber-Lang, Flierl, & Ward, 2009). 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine were administered intraperitoneally as anesthesia, and the procedures indicated in Table 1 were performed respectively. Briefly, the rats were placed in the supine position, and an incision approximately 2 cm long was made in the midline of the abdomen. In the abdomen, the cecum was carefully isolated, and its distal 2/3 part was ligated with 4/0 silk thread so that the intestinal passage could still be provided. Then, the connected part was perforated by piercing it twice with a sterile 21-gauge needle. A small amount of stool was expelled to ensure perforation. The cecum was placed inside the abdomen, and the abdominal incision was closed by primary suture. The procedure was terminated with sterile dressing in the operation area (Deitch, 1998; Demirbilek et al., 2006; Lee, Emala, Joo, & Kim, 2007; Lewis, Seymour, & Rosengart, 2016; Salkowski, Detore, Franks, Falk, & Vogel, 1998). In the control group (Negative Control Group), only the abdominal incision was made without applying cecum ligation and perforation with a similar procedure and then sutured and closed (Sham Operation). To ensure fluid resuscitation, all rats administered 1 ml of warmed sterile saline (SF) subcutaneously (s.c.) immediately after the operation. The first injections were made 2 hours after the surgical operation and continued for 4 days with an interval of 12 hours, and the experiment was terminated after the sixth injection (approximately 72 hours after the surgical operation).




**Treatment of sepsis model**

Table 1 shows the groups and information about the groups.

| | **Table 1** group information | **Table 1** group information |
|---|---|---|
| 1 | Negative Control Group (n=8) | This group had undergone a surgical incision called "Sham Operation" to mimic the same after-surgical effects. The rats did not have any treatment, only were given a Saline solution (0.9% NaCl) every 12 hours through 72 hours. |
| 2 | Positive Control Group (n=8) | This group had undergone a surgical procedure which is called the Cecal Ligation & Puncture (CLP) to mimic sepsis syndrome. The rats did not have any treatment, only were given a Saline solution (0,9% NaCl) every 12 hours through 72 hours. |
| 3 | Fluoxetine Group (n=8) | The CL&P method was applied, and rats received 5 mg/kg Fluoxetine (FLU) intraperitoneally every 12 hours through 72 hours (10 mg/kg/day). |

| | **Table 1** group information | **Table 1** group information |
|---|---|---|
| 4 | Imipenem Group (n=8) | The CL&P method was applied, and rats received 60 mg/kg Imipenem (IMP) intraperitoneally every 12 hours through 72 hours (120 mg/kg/day). |
| 5 | Combined Group (n=8) | The CL&P method was applied, and rats received 5mg/kg Fluoxetine (FLU) and 60 mg/kg Imipenem (IMP) intraperitoneally every 12 hours through 72 hours (10 mg/kg/day and 120 mg/kg/day). |

## Collection of blood and tissue

72 hours after the operation, the rats were anesthetized, and the liver, kidney, and intestinal tissues were removed. Some of the tissues harvested were frozen at -80°C for biochemical analysis, while the others were fixed in a 10% formaldehyde solution to be used for histopathological studies. Blood samples collected into tubes without anticoagulation and $K_2$EDTA were centrifuged at 3000 rpm for 10 minutes to obtain serum and plasma. The separated serum and plasma were stored in a -80°C freezer until the day of analysis.

## Blood and tissue analyzes

### Blood culture

Microbiological growth in blood was detected in blood culture. After the blood, cultures were obtained in a sterile manner from rats according to BD Biosciences protocol, and the data after performing of the blood cultures were read with a nonradiometric continuous monitoring system (The BD BACTEC FX blood culture system; Bezmialem Vakif University Hospital Medical Microbiology Department).

### Serum and tissue preparation

The liver, kidney, and intestinal blood tests, total antioxidant capacity, total oxidant status, total antioxidant status, total thiol, native thiol, and inflammatory markers, and growth factors were measured from the serum samples separated at the end of the experiment.

Tissues were homogenized with ceramic balls for 1 minute with 1x Phosphate-buffered saline (PBS) buffer and centrifuged at 12,000 g for 30 minutes at temperature 4degC. After the centrifugation step, the supernatant was taken, and protein concentration was determined with the Bradford assay (Coomassie Plus (Bradford) Protein Assay, Thermo Scientific).

### Routine sepsis tests

The Procalcitonin (PCT) levels in serum were measured by the immunoassay method (Architect i1000sr; Abbott Laboratories, Abbott Park, IL, USA). The concentration of high-sensitivity C-reactive protein (hs-CRP) was measured by an immunoturbidimetric assay using the hs-CRP kit (Architect C16000; Abbott Laboratories, Abbott Park, IL, USA). Lactate levels were measured using a blood gas analyzer.

### Inflammation markers

The investigated blood samples were measured by the spectrophotometric method used by commercially purchased Enzyme-linked immunosorbent assays (ELISA) (Elabscience, Texas, USA) to measure myeloperoxidase activity (MPO), tumor necrosis factor (TNF), interleukin-1β (IL-1β), interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP) levels. Absorbance values at 450 nm wavelength were recorded on a microplate reader (ThermoScientific Varioskan Flash Multimode Reader).

### Oxidative stress markers

Total antioxidant status measurement was done according to the method developed by Erel et al. (Erel, 2004). The principle of the method is based on reducing the dark blue-green ABTS radical of the antioxidants in the example to the colorless ABTS form. As a standard, ascorbate was used, and results for serum/plasma were given as mM ascorbate equivalent and mM ascorbate equivalent/mg protein for tissue.

Total oxidant status was performed with another method developed by Erel et al. (Erel, 2005). The principle of the method is to oxidize the ferrous ion chelator complex of the oxidants in the sample to ferric ions and to form the color of these ferric ions with chromogen in an acidic medium. As a standard, μM $H_2O_2$was used, and results for serum/plasma were given as μM $H_2O_2$ equivalent, μM $H_2O_2$ equivalent/mg protein for tissue.

For the measurement of thiol-disulfide homeostasis, the automated method developed by Erel et al. was used (Erel & Neselioglu, 2014). Total thiol (-SH + –S-S–) and native thiol (-SH) concentrations in the samples were measured using Ellmann and modified Ellmann reagents. - SH content was removed from –SH + –S - S– content, and half of this difference was calculated, and the number of dynamic disulfide bonds (-S-S-) was determined.

### Mononuclear leukocytes isolation

For the comet assay was mononuclear leukocyte isolation was performed by the use of Histopaque 1077 (Sigma Aldrich). 1 mL of whole heparinized blood was gently layered over 1 mL Histopaque and centrifuged for 25 min at 2100 rpm at room temperature. The interface band containing mononuclear leukocytes was washed with phosphate-buffered saline (PBS) and then collected after 10 min centrifugation at 2500 rpm.

### DNA damage

Microscope slides were coated with 1.0% hot (45°C) normal melting point agarose (NMA) agarose prepared in phosphate-buffered saline. Then the microscope slides were covered with a coverslip at 4°C for at least 5 minutes until the agarose solidified. After the removal of the coverslips, the mixture of 85 μL of 0,6% low melting agarose (LMA) and 15 μL isolated mononuclear leukocyte were placed on pre-coated slides of 1% normal melting agarose (NMA). The slides were allowed to solidify for 10 min at 4°C. The slides were immersed in the freshly prepared cold (4°C) lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris-HCl, and pH 10–10.5 with 1% Triton X-100 added) overnight. After the overnight, the slides were incubated for 40 minutes in the electrophoresis containing running buffer solution (0.3 mol/L NaOH and 1 mmol/L Na2 EDTA, pH .13) at a temperature of 4°C in the dark. Electrophoresis was implanted for 35 minutes at 26 Volt, 300mA in an electrophoresis tank. After electrophoresis, the slides were stained with ethidium bromide (2 μL/mL $H_2O$, 70 μL/slide) and visualized using an epifluorescence-equipped 200x magnification fluorescence microscope (Leica DM 1000, Solms, Germany) equipped with a rhodamine filter (excitation wavelength of 546 nm and a barrier of 580 nm). We scored with a fluorescence microscope (Leica DM 1000, Solms, Germany) using the Comet assay IV software (Perceptive Instruments, Suffolk, UK) (Guler et al., 2020).

### Statistical Analysis

All experiments were done in triplicate, and the obtained results were expressed as mean value ± standard deviation (Mean ± SD). By using the analysis of variance (One-way ANOVA) was a statistical evaluation performed. The differences with a probability value were statistically significant accepted ($p < 0.05$). The statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 25.

## 3. Results

### Oxidative Stress status

#### Oxidative Stress status in serum

Total oxidant status (TOS) and Oxidative stress index (OSI) levels began to decrease by combined treatment of fluoxetine 10 mg/kg per day and imipenem 120 mg/kg per day. The changes in oxidative stress biomarker levels of serum in different groups are given in Figures 2A and 2B.

The oxidative stress index levels were significantly reduced by the experimental sepsis model mostly after combined treatment with fluoxetine and imipenem ($p<0.001$). It decreases by 51.57% to 21.53±11.32 arbitrary units (AU) in the fluoxetine treatment group, 60.30±6.44 arbitrary units in the imipenem treatment group by 72.56% to 12.20±1.49 arbitrary units in the combined group of fluoxetine and imipenem compared to the positive control.

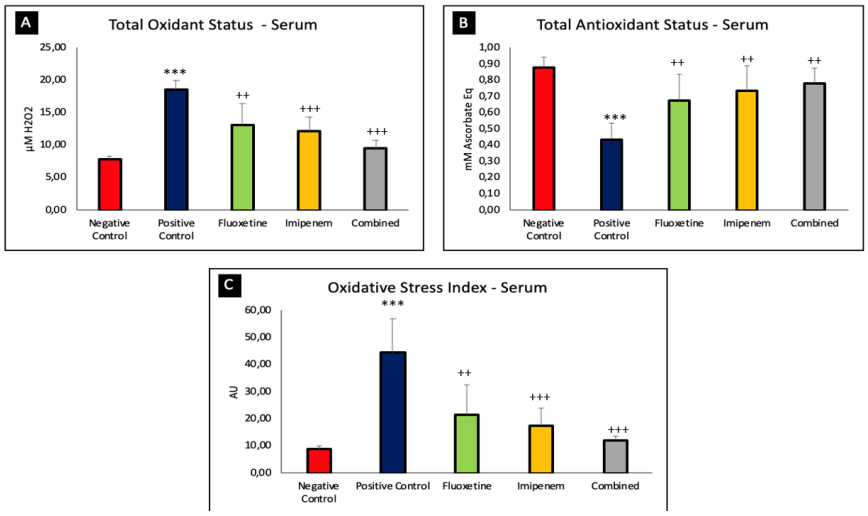



**Figure 2** A) Serum total oxidant level: TOS increased statistically significantly in the positive control group compared to the negative control group (healthy). B) Serum total antioxidant level: TAS levels decreased statistically significantly in the positive control group compared to the negative control group (healthy). In addition, changes in all treatment groups are statistically significant compared to the positive control group. C) Serum oxidative stress index: The positive control group OSI increased statistically significantly compared to the negative control group (healthy). Also, the changes in all treatment groups are statistically significant compared to the positive control group. Data are expressed as the mean ± standard deviation for five to eight animals per group (average ± SD). + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

The total Thiol (TT) levels decreased statistically significantly by 42.00%, the native thiol (NT) levels by 81.00% on average in the positive control (experimental sepsis) group compared to the negative control group

(healthy group). The TT and NT levels of treatment groups changed statistically significantly compared to the positive control group. And Myeloperoxidase (MPO) enzyme decreased significantly by 64.83% in the combined (fluoxetine and imipenem) group compared to the positive control (Figure 3).

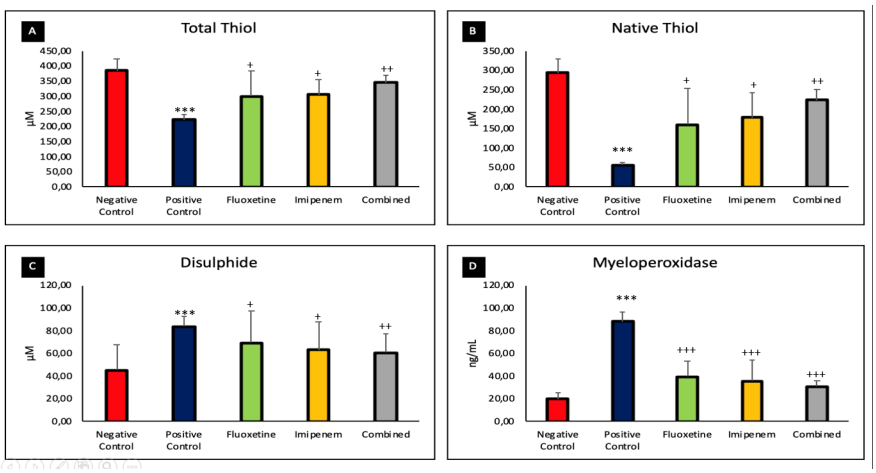



**Figure 3** A) Serum total thiol level: compared to the negative control group (healthy), the positive control group decreased statistically significantly. Also, the changes in all treatment groups are statistically significant compared to the positive control group. B) Serum native thiol level: The positive control group NT levels decreased statistically significantly compared to the negative control group (healthy). C)Serum disulfide level: The positive control group DS levels increased statistically significantly compared to the negative control group (healthy). D) And Myeloperoxidase (MPO) enzyme increased significantly compared to the negative control group. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD). + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

### Oxidative Stress status in tissues

Oxidative stress indicators were investigated in the liver, kidney, and ileum of the experimental groups to determine the mechanisms of liver, kidney, and ileum damage caused by sepsis.

Oxidative stress status in liver

Total oxidant status (TOS) and Oxidative stress index (OSI) levels began to decrease by treatment groups: fluoxetine 10mg/kg per day, imipenem 120 mg/kg per day, and combined treatment of fluoxetine 10 mg/kg and imipenem 120 mg/kg per day. The changes in oxidative stress biomarker levels of liver tissue in different groups are given in Figures 4A and 4B. And in figure 4C, the treatment group fluoxetine decreased by 52% to 21.22±19.08 arbitrary units, in the imipenem group decreased by 63% to 16.49±11.30 arbitrary units and the combined group 83% to 7.73±2.52 arbitrary units compared to the positive control group ($p<0.05$).

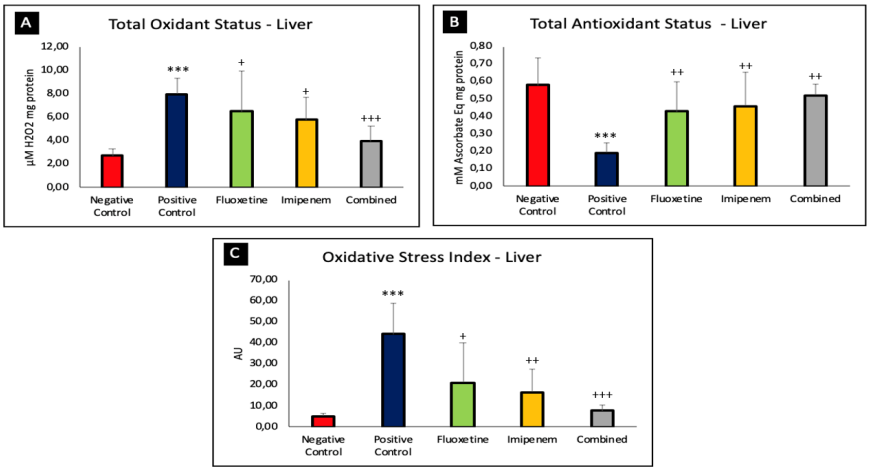



**Figure 4** Indicators of oxidative stress in rat liver tissues following the experimental sepsis model. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD). A) Liver total oxidant level: TOS increased statistically significantly in the positive control group compared to the negative control group (healthy). B) Liver total antioxidant level: TAS levels decreased statistically significantly in the positive control group compared to the negative control group (healthy). In addition, changes in all treatment groups are statistically significant compared to the positive control group. C) Liver oxidative stress index: The positive control group OSI increased statistically significantly compared to the negative control group (healthy). Also, the changes in all treatment groups are statistically significant compared to the positive control group. + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

Oxidative stress status in kidney

Total oxidant status (TOS) and Oxidative stress index (OSI) levels began to decrease by treatment groups: fluoxetine, imipenem, and combined treatment of fluoxetine and imipenem.

The changes in oxidative stress biomarker levels of kidney tissue in different groups are given in Figures 5A and 5B. And in figure 5C, the treatment group fluoxetine decreased by 31% to 35.62±27.57 arbitrary units, in the imipenem group decreased by 33% to 34.51±28.38 arbitrary units and the combined group 79% to 10.85±2.64 arbitrary units compared to the positive control group ($p < 0.05$).

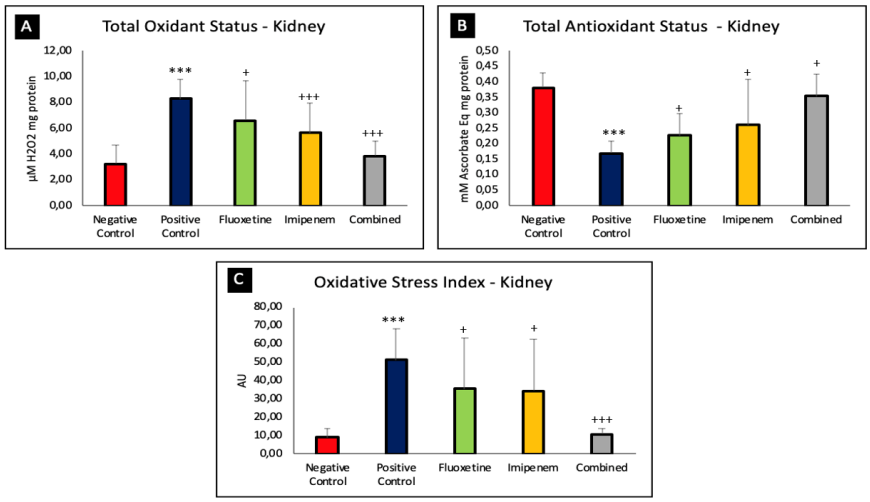


**Figure 5** Indicators of oxidative stress in rat kidney tissues following the experimental sepsis model. Data are expressed as average ± standard deviation (n=5-8). A) Kidney total oxidant level: TOS increased statistically significantly in the positive control group compared to the negative control group (healthy). B) Kidney total antioxidant level: TAS levels decreased statistically significantly in the positive control group compared to the negative control group (healthy). In addition, changes in all treatment groups are statistically significant compared to the positive control group. C) Kidney oxidative stress index: The positive control group OSI increased statistically significantly compared to the negative control group (healthy). Also, the changes in all treatment groups are statistically significant compared to the positive control group. + = $p < 0.05$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

Oxidative stress status in the ileum

Total oxidant status (TOS) and Oxidative stress index (OSI) levels began to decrease by treatment groups fluoxetine, imipenem, and combined treatment of fluoxetine and imipenem.

The changes in oxidative stress biomarker levels of kidney tissue in different groups are given in Figures 6A and 6B. In figure 6C, the treatment group fluoxetine decreased by 66% to 25.27±24.86 arbitrary units in the imipenem group decreased by 79% to 15.78±10.84 arbitrary units and combined group by 90% to 7.56±3.07 arbitrary units compared to the positive control group ($p<0.05$).

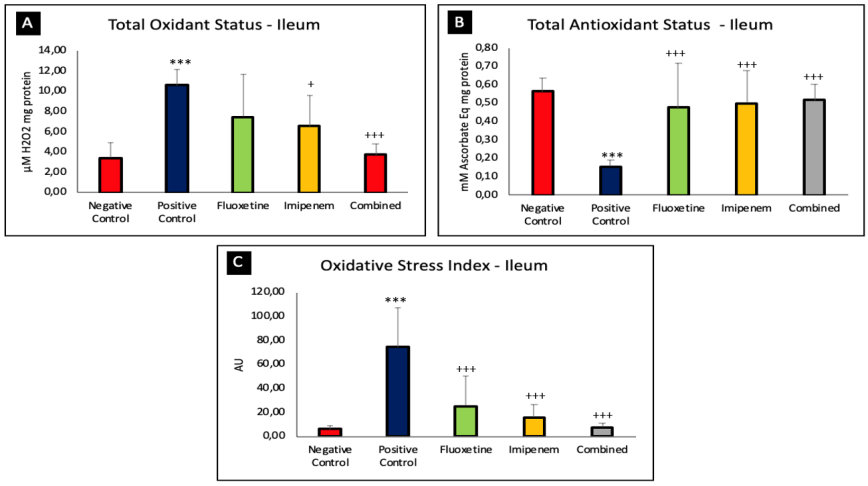



**Figure 6** A) Liver total oxidant level: TOS increased statistically significantly in the positive control group compared to the negative control group (healthy). B) Liver total antioxidant level: TAS levels decreased statistically significantly in the positive control group compared to the negative control group (healthy). Besides, changes in all treatment groups are statistically significant compared to the positive control group. C) Liver oxidative stress index: The positive control group OSI increased statistically significantly compared to the negative control group (healthy). Also, the changes in all treatment groups are statistically significant compared to the positive control group. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD). + = $p < 0.05$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

## Inflammatory Parameters

### Inflammatory parameters in serum

Inflammatory biomarkers levels in all groups can be seen in figure 7; when compared to the positive control group, pro-inflammatory cytokines IL-1β, IL-6, and TNF-α levels in serum were significantly lower in all treatment groups (fluoxetine, imipenem, and combined). These cytokine levels were significantly reduced by an experimental sepsis model after combined treatment with fluoxetine and imipenem ($p<0.05$). Proinflammatory cytokines IL-1β decrease by 63.11% to 312.80±34.94 pg/mL, IL-6 by 83.39% to 3.80±0.72 ng/mL, and TNF-α by 79.52% to 40.60±11.56 ng/L in the combined group of fluoxetine and imipenem compared to the positive control.

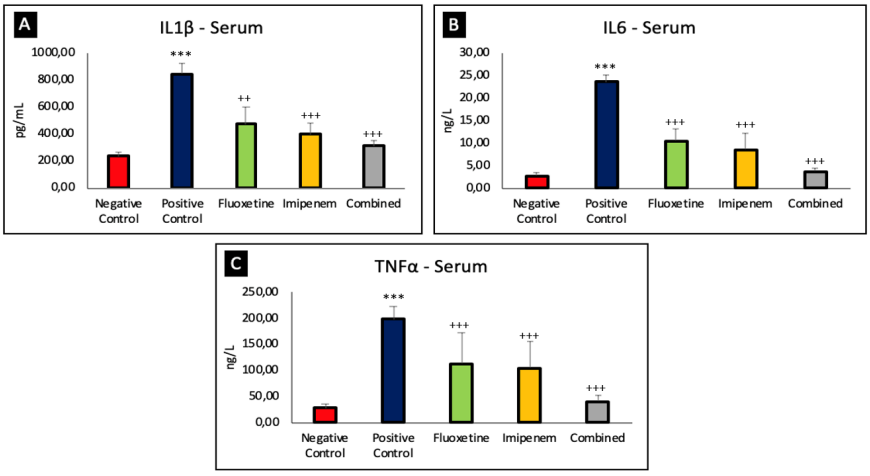



**Figure 7** Effect of fluoxetine and Imipenem treatment on proinflammatory cytokines in the serum of rats. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD). A) Serum IL-1β level: IL-1β decreased statistically significantly in the treatment groups compared to the positive control group. B) IL-6 level: IL-6 levels decreased statistically significantly in the positive control group compared to the positive control group. C) TNF-α level changes in all treatment groups are statistically significant compared to the positive control group. ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

The hs-CRP and PCT levels in all groups are given in figure 8. By combined group of fluoxetine and imipenem the hs-CRP decreased by 87,24% to 0,27±0,07 ng/mL and Procalcitonin by 96.64% to 49.77±13.68 ng/L compared to positive control (hs-CRP: 7.95 ng/mL and PCT: 389.90 ng/mL).

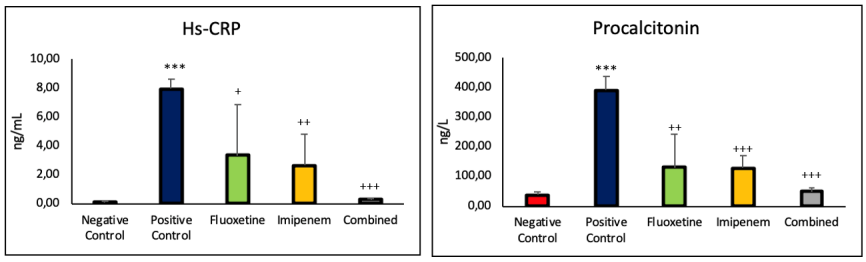



**Figure 8** hs-CRP and Procalcitonin levels of serum in rats. The negative control is a group without experimental sepsis. The positive control is a group with an experimental sepsis model. The further groups

are treated with 10 mg/kg fluoxetine, 120 mg/kg imipenem, and a combined group with fluoxetine and imipenem. Data are expressed as the mean ± standard deviation for five to eight animals per group (mean ± SD). + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

After treatment with fluoxetine, imipenem, and the combination (Fluoxetine and Imipenem), the monocyte chemoattractant protein (MCP-1) and lactate levels in serums were reduced ($p<0.05$). The Lactate and MCP-1 levels in all groups can be seen in table 2. The lactate levels of the fluoxetine treated group decreased by 46%; the imipenem treated group by 53%, and the combined group by 76% compared to the positive control. The MCP-1 levels of the fluoxetine treated group decreased by 25%; the imipenem treated group by 31%, and the combined group by 43% compared to the positive control.

**Table 2** The results Lactate and MCP-1 in the serum of the rat. SD means standard deviation. + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).

| | | Negative Control n=7 | Positive Control n=7 | Fluoxetine 10 mg/kg n=7 | Imipenem 120 mg/kg n=7 | Combined 10mg/kg+120 mg/kg n=7 |
|---|---|---|---|---|---|---|
| **Lactate** ng/µL | Average±*SD* | 1.51±0.30 | 8.68±0.63 *** | 4.65±3.05 + | 4.11±2.17 ++ | 2.05±0.42 +++ |
| **MCP-1** ng/L | Average±*SD* | 202.59±29.56 | 320.49±93.27 *** | 238.38±45.51+ | 221.99±48.39 ++ | 181.56±35.67 +++ |

**Inflammatory parameters in tissues**

The IL-1β, IL-6, and TNF-α production capacity of the groups was determined by measuring the cytokine levels in the liver, ileum and kidney tissues after a surgical procedure of 72 hours.

Inflammatory biomarker levels in all tissue groups can be seen in figure 9. These cytokine levels were significantly reduced by an experimental sepsis model after combined treatment with fluoxetine and imipenem ($p<0.05$). Proinflammatory cytokine IL-1β in the liver decreased by 61% to 653.43±80.15 pg/mL mg protein, in the ileum by 56% to 859.65±48.20 pg/mL mg protein, and in the kidney by 27% to 582.50±63.06 pg/mL mg protein compared to the positive control. The IL-6 cytokine in the liver decreased by 68% to 10,94±1,89 ng/mL mg protein, in the ileum by 67% 13.34±3.45 ng/mL mg protein, and in the kidney by 49% to 12.78±0.86 ng/mL mg protein compared to the positive control. And the proinflammatory cytokine TNF-α decreased in the liver by 49.14% to 207.16±21.06 ng/L mg protein, in the ileum by 66% to 186.33±32.87 ng/L mg protein, and in the kidney, by 52% to 159.31±45.89 ng/L mg protein in the combined group of fluoxetine and imipenem compared to the positive control.

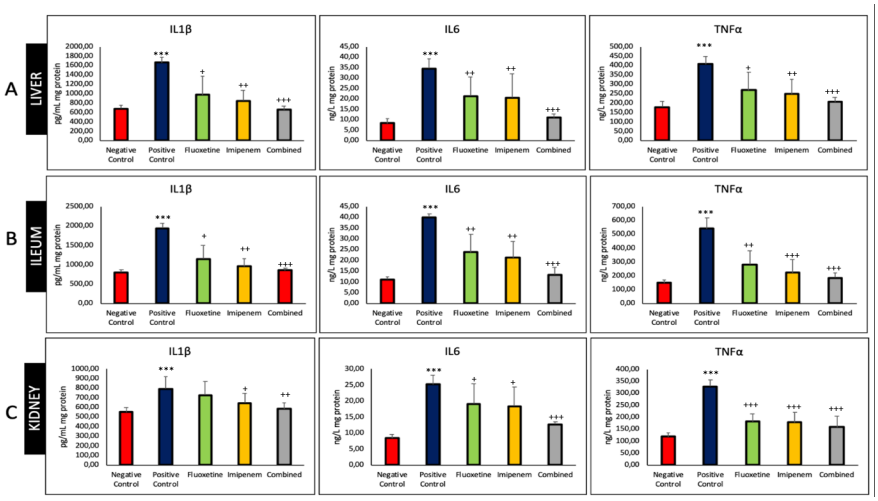



***Figure 9*** *Effect of Fluoxetine and Imipenem treatment on proinflammatory cytokines in tissues of rats. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD). A) Liver tissue IL-1, IL-6 and TNF- levels: decreased statistically significantly in the treatment groups compared to the positive control group. B) Ileum tissue IL-1, IL-6 and TNF- levels: decreased statistically significantly in the positive control group compared to the positive control group. C) Kidney tissue IL-1, IL-6 and TNF- levels decrease in all treatment groups are statistically significant compared to the positive control group. + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).*

## Blood culture

Bacteremia can cause a systemic inflammatory reaction syndrome and can lead to severe life-threatening sepsis. Blood culture was taken to identify the bacteria in the blood—table 3 shows which bacteria have been affected per group. No bacteria were present in the negative control group and the combined group. In the positive group, bacteria*E. coli* , *S. viridans,* and *S. haemolyticus* were present. And in the Imipenem treatment group, *E. coli* , *E. faecium,* and *R. pneumotropicus* were present.

**Table 3** Bacteria identification of bloodstream infection in rats.

| n | Negative Control | Positive Control | Fluoxetine | Imipenem | Combined |
|---|---|---|---|---|---|
| **1** | Negative | *E. coli* | Negative | Negative | Negative |
| **2** | Negative | *E. coli* | *E. coli* | Negative | Negative |
| **3** | Negative | *E. coli* | Negative | *E. coli* | Negative |
| **4** | Negative | *E. coli* | *E. coli* | *E. faecium* | Negative |
| **5** | Negative | *S.viridans, S. haemolyticus* | Negative | *E. faecolis* | Negative |
| **6** | Negative | *E. coli* | *E. coli* | Negative | Negative |
| **7** | Negative | *E. coli* | Negative | *R. pneumotropicus* | Negative |

## DNA damage

After the isolation of mononuclear leukocytes, the DNA damage of the obtained serum after the treatment was determined by Comet assay. The Comet assay was performed to find the level of DNA damage in the

rats of different treatments, which can be seen in Figures 10 and 11.

The damaged DNA nuclei had a bright head and a tail. The core with undamaged DNA appears round. A longer DNA migration smear (comet tail) in positive control can be seen in figure 11B. The DNA damage in the positive group is significantly higher than in the control group. DNA damage of combination group fluoxetine and imipenem ranged by 10.65% in the experimental sepsis model, which compared to the positive control of 68.15%, see figure 10.

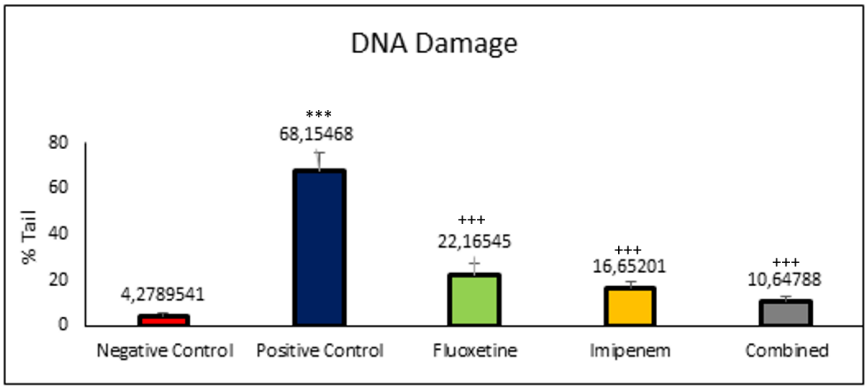
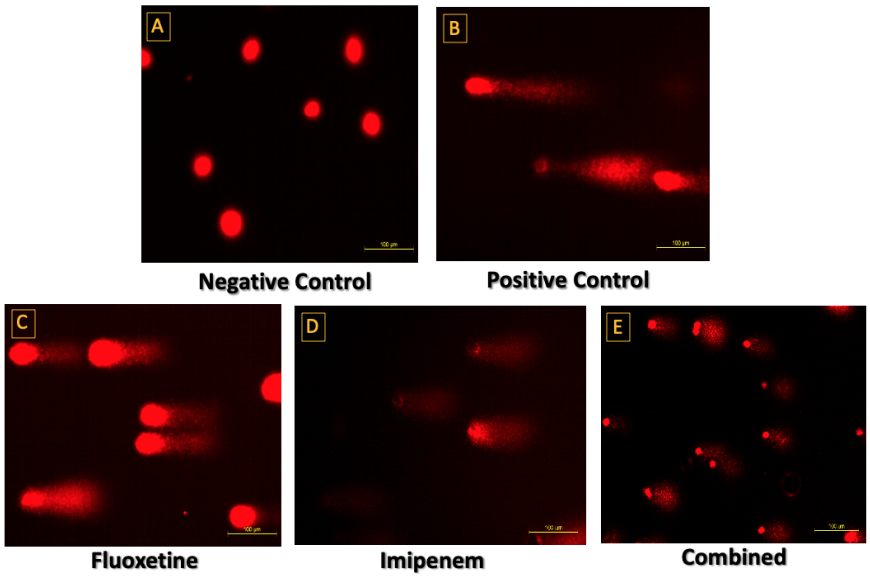



**Figure 10** Comet tail length after treatment; fluoxetine, imipenem, and combination (fluoxetine +iImipenem) groups. DNA Damage reduced ($p<0.05$). + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$. *** = $p < 0.001$ compared with the positive group (sepsis).




**Supplementary Figure 11** Comet Assay analysis of DNA damage in rats versus healthy controls. Figures A-D give examples of images typical for A) negative control group Sham operation without CLP and treatment, B) positive control without treatment, C) treatment group with fluoxetine 10mg/kg per day, D) treatment group with imipenem 120 mg/kg per day, and E) Combined group treatment with fluoxetine 10mg/kg and imipenem 120 mg/kg per day. Comet-patterns typical for healthy controls (A) show that chromosomal DNA is localized mainly to heads of comets (intact DNA). In contrast, images B, C, D, and E demonstrate damaged DNA.

# 4. Discussion

In this research, 40 rats were equally divided into five groups. The first group was a negative control, group 2 the positive control, group 3 was treated with fluoxetine 10 mg/kg per day, group 4 treated with imipenem antibiotic 120 mg/kg per day, and group 5 treated with a combination of fluoxetine 10 mg/kg and imipenem 120 mg/kg per day through 4 days. Polymicrobial sepsis was generated by the CLP method (Liu et al., 2011). After the treatment, the blood samples and tissues were studied for HsCRP, pro-calcitonin (PCT), lactate, myeloperoxidase activity (MPO), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) were measured using ELISA methods. Oxidative stress markers total thiol (TT), native thiol (NT), total oxidant status (TOS), and total antioxidant status (TAS) were studied by different photometric methods.

Blood culture was taken to identify the bacteria in the whole blood. Bacteremia can cause a systemic inflammatory reaction syndrome and can lead to severe life-threatening sepsis.

No bacteria were present in the negative control group; this was also the intention because no sepsis model has been created in this group. In a positive group whereby CLP is used, bacteria *E. coli* , *S. viridans,* and *S. haemolyticus* were present in the blood of rats. In the Imipenem treatment group, *E. coli* , *E. faecium,* and*R. pneumotropicus* were present. The bacteria *S. viridans*and *S. haemolyticus* are commensal in the skin, and *E. faecium* and *R. pneumotropicus* are commensal in the gastrointestinal tract and urogenital tracts. These bacteria may have been detected through contamination during the surgical procedure. In the combined group fluoxetine and imipenem, there were no bacteria observed. This can indicate that combined group treatment will be effective on sepsis.

Oxidative stress has been defined as a disturbance in the balance between oxidants and antioxidants, which may lead to damage and tissue injury (Halliwell, 1994). Therefore, oxidative stress contributes to the sepsis process. Oxidative stress markers total thiol (TT), native thiol (NT), total oxidant status (TOS), and total antioxidant status (TAS) were studied by different photometric methods. The TT and NT levels of treatment groups changed statistically significantly compared to the positive control group. In our study, we determined the oxidative stress according to the oxidative stress index. The highest oxidative stress index level of serum was by positive control. The oxidative stress index level was significantly reduced by the experimental sepsis model by first fluoxetine and then imipenem. But it decreased mostly after combined treatment with fluoxetine and imipenem. In addition, oxidative stress indicators were investigated in the liver, kidney, and ileum of the experimental groups to determine the mechanism of liver, kidney, and ileum damage caused by sepsis. Liver dysfunction is frequently seen in early sepsis results from altered hepatocellular functions (Halliwell, 1994). In sepsis, kidney damage can be observed along with liver damage. The order of kidney and liver functions is interconnected. Kidney disease occurs in 20-25% of liver patients (Gonwa & Wadei, 2013). Total oxidant status (TOS) and Oxidative stress index (OSI) levels in the liver began to decrease by all treatment groups: fluoxetine, imipenem, and combined treatment of fluoxetine and imipenem. From the results, OSI levels were highest decreased by the combined treatment.

A study found that apoptosis and necrosis increase during severe sepsis and septic shock (Bahar, Elay, Başkol, Sungur, & Donmez-Altuntas, 2018). After the isolation of mononuclear leukocytes, the DNA damage of the obtained serum after the treatment was determined by Comet assay. The Comet assay was performed to find the level of DNA damage in obtained blood from rats.

Our results indicated that the lowest damage was observed by the combination of fluoxetine and imipenem in the experimental sepsis model.

The lactate levels rising as a result of sepsis are also mentioned in the literature. In the study in patients with sepsis in intensive care, lactate levels were statistically higher in deceased patients than in convalescent patients (Bayır, Yıldız, Erkuran, & Koçoğlu, 2015). A decrease of at least 20% in lactate value is an important in-patient prognostic factor (Rabello Filho et al., 2016). It appears from the results, the lactate levels in serum decreased in order fluoxetine, imipenem, and combined group compared to the positive control. All

three treatment groups decreased lactate levels by the sepsis model. However, the best result is obtained from the combined group of Fluoxetine and Imipenem.

From the results of the inflammatory response panels can be seen that the positive control group in which sepsis has been created has a high percentage of TNF-α, IL-1β, IL-6, MCP-1, and hs-CRP. This was also the intention because, by the sepsis, the damaged tissue cells are recognized by macrophage receptor Toll-like receptor (TLR) and leads to the production of proinflammatory cytokines TNF-α, IL-1β and IL-6, and MCP-1. The IL-6 stimulates the liver to produce hs-CRP (Khushboo & Sharma, 2017). The most commonly used sepsis biomarkers in the routine lab are Procalcitonin (PCT) and high-sensitivity C-reactive protein (hs-CRP). The role of hs-CRP during acute inflammation is to bind the phospholipid components of microorganisms and hereby damage host cells. It is commonly used to screen for early sepsis (Khushboo & Sharma, 2017). From the results of research, the hs-CRP and Procalcitonin in serum were highly decreased by the combined group of fluoxetine and imipenem compared to the positive control. Myeloperoxidase (MPO) enzyme was evaluated as an indicator of the inflammatory process. In our results, a significant increase was detected in the positive group compared to the negative group. And the mostly decreased percentage was in the combined (fluoxetine and imipenem) group compared to the positive control.

In the first period of inflammatory cytokines, TNF-α, IL-1β, IL-6 play the leading role, the leukocytes are the major production source. One of the most interesting studies explaining that TNF α is responsible for organ injury and death in sepsis was performed by Waage et al. in 1987. This study states that sepsis patients have high TNF-α levels, which is a turning point in understanding sepsis (Waage, Halstensen, & Espevik, 1987). Many studies have now confirmed that there is a close relationship between increased plasma TNF α levels and increased mortality (Gogos, Drosou, Bassaris, & Skoutelis, 2000; Terregino, Lopez, Karras, Killian, & Arnold, 2000).

In our study, we found that pro-inflammatory cytokines IL-1β, TNF-α, and IL-6 levels in serum are remarkably decreased in the experimental sepsis model, mainly after combined treatment with fluoxetine and imipenem.

Fluoxetine is known to act as an inhibitor of efflux pumps in human cells. The inhibition of these pumps appears to be an attractive strategy at a time when shrinking the number of antibiotics (Munoz-Bellido et al., 2000). Also, fluoxetine inhibits the production of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO), which are induced by lipopolysaccharide (LPS) of gram-negative bacteria (Charles et al., 2017; Liu et al., 2011). Fluoxetine was found to suppress the number of inflammation-related cells and TNF-α release from monocytes in the septic shock animal model (Roumestan et al., 2007). In a study, it was shown that the association of fluoxetine with antibiotic gentamicin or erythromycin showed synergistic effects. Fluoxetine increased the effectiveness of the antibiotics (Roumestan et al., 2007). This also corresponded to our results. In our study, the rats were also treated with an antibiotic primarily used in sepsis, namely imipenem. This antibiotic belongs to the Carbapenem family and works by disrupting the cell wall of the bacteria. Fluoxetine and imipenem drugs can have direct antimicrobial activity, increase the efficiency of an antibiotic when given together (Munoz-Bellido et al., 2000).

# 5. Conclusion

Our study shows that fluoxetine has anti-inflammatory and antioxidant effects and, its combined therapy with imipenem shows positive synergistic effects against the experimental sepsis model. Therefore, fluoxetine can be useful for the treatment of sepsis together with imipenem to ameliorate sepsis in our rat model.

# Recommendation

As a result of our study, the combined therapy of fluoxetine and imipenem has been shown to have an anti-inflammatory and antioxidant effect as a result of its synergistic effects in the *in-vivo*sepsis model.

Considering the *in-vivo* results in our study, it was thought that fluoxetine may be an option in the combined treatment with imipenem. Considering that the drug is given only intravenously in the treatment of sepsis, the development of intravenous formulations of fluoxetine can be useful for treatment.

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Conflict Of Interest: The authors declare no conflicts of interest.

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

Declaration of Transparency and Scientific Rigour: This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical

research as stated in the BJP guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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