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Examination of antimicrobial effect of fluoxetine in experimental sepsis model: An in vivo study

Ahsen Cakir[1] | Kubra Bozali[2,3] | Mert Celikten[4,5] | Eray Metin Guler[3,6] | Ebru Sahan[7] | Bulent Durdu[8] | Bilge Sumbul[9] | Abdurrahim Kocyigit[6]

[1]Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

[2]Faculty Life Sciences and Environmental Technology, Avans University of Applied Science, Breda, The Netherlands

[3]Department of Medical Biochemistry, Faculty of Hamidiye Medicine, University of Health Sciences Turkey, Istanbul, Turkey

[4]Department of Scientific Research Projects, Experimental Application and Research Center, Bezmialem Vakif University, Istanbul, Turkey

[5]Department of Anatomy, Medipol Institute of Health Sciences, Istanbul, Turkey

[6]Department of Medical Biochemistry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

[7]Department of Psychiatry, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

[8]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

[9]Department of Medical Microbiology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

Correspondence

Kubra Bozali, Faculty Life Sciences and Environmental Technology, Avans University of Applied Science, Breda, The Netherlands.
Email: kubrabozalii@gmail.com

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Abstract

Since most infectious diseases can develop into sepsis, it is still a major medical problem. Some in-vivo studies showed promising properties of fluoxetine in the treatment of infections. This study aims the antimicrobial effect of fluoxetine on the inflammatory process used in the treatment of sepsis-modeled rats. Besides, to investigate the efficacy of fluoxetine on modifying the antibiotic effect of imipenem in the inflammatory response. An experimental sepsis model was divided into negative control, positive control, fluoxetine 5 mg/kg, imipenem 60 mg/kg, and combined (fluoxetine; imipenem). Procalcitonin (PCT), high-sensitivity C-reactive protein (hs-CRP), lactate, myeloperoxidase activity (MPO), the inflammation markers interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alfa (TNF-α), and monocyte chemoattractant protein-1 (MCP-1) levels were measured by enzyme-linked immunosorbent assay method. Oxidative stress markers, total oxidant status (TOS), total antioxidant status (TAS), total thiol (TT), and native thiol (NT) were measured using photometric methods. Oxidative stress index (OSI) was calculated according to TAS and TOS levels. The statistical analysis was performed by Statistical Package for Social Sciences version 22.0. After treatment with fluoxetine, imipenem, and combined groups, IL-1β, IL-6, TNF-α, MPO activity, MCP-1, hs-CRP, PCT, lactate, and the oxidative stress markers OSI, and disulfide levels were decreased ($p < 0.05$). The TT, NT, and TAS levels significantly statistically increased ($p < 0.05$). This research demonstrates that fluoxetine has effects as anti-inflammatory and antioxidant, and the combined treatment with antibioticum imipenem indicates positive synergistic effects in the experimental sepsis model.

KEYWORDS

antidepressive, fluoxetine, imipenem, inflammation, sepsis

Abbreviations: CLP, cecal ligation and puncture; DAMPs, damage-associated molecular patterns; hs-CRP, high-sensitivity C-reactive protein; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MPO, myeloperoxidase activity; NO, nitric oxide; NT, native thiol; OSI, oxidative stress index; PAMPs, pathogen-associated molecular patterns; PCT, procalcitonin; SSRI, selective serotonin reuptake inhibitors; TAS, total antioxidant status; TLRs, toll-like receptors; TNF-α, tumor necrosis factor-alpha; TOS, total oxidant status; TT, total thiol.

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Ahsen Cakir and Kubra Bozali contributed equally to this study.

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1 | BACKGROUND

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.[1] Sepsis is caused by bacteria, viruses, fungi, parasites, and a noninfectious event such as severe trauma. The microorganism that enters the body can lead to various cases, from infection and bacteremia to sepsis and septic shock leading to death.[2]

When used wisely and timely, antibiotics cater as a great tool in combatting the attacks of microorganisms. But antibiotics alone are not enhancements, which will improve the overall septic appearance. At the same time, it is known that antibiotics trigger the release of bacterial cell wall components, which partake in severe inflammation that leads to sepsis in the body. Unfortunately, despite the advantages of antibiotics, there is one more drawback that has become a bigger problem lately. In neonatal sepsis deaths, more than 30% are based upon antibiotic resistance. There is an urgent need to develop alternative approaches for the treatment of antibiotic resistance in sepsis.[3] This need has directed researchers to several nonantibiotic products and drugs. It has been shown that several nonsteroidal anti-inflammatory drugs (NSAIDs), mucolytic agents, calcium channel blockers, and proton pump inhibitors have some influence on the physiology and viability of microorganisms.

Antidepressants help reduce symptoms of depressive disorders by altering chemical imbalances of neurotransmitters located in vesicles found in nerve cells of the brain. Researchers mainly focused on whether depression can be treated with nonsteroidal anti-inflammatory drugs when the hippocampal inflammation hypothesis was first put forward. When the other side of the coin started to be considered, the question arose whether antidepressants that treat depression have a therapeutic effect in other systemic inflammation conditions.

After the antimicrobial effects of chlorpromazine, a dopamine antagonist was found in 1959, and antidepressants were also included in antimicrobial effective nonantibiotic drugs. As the new antidepressants sertraline, fluoxetine, and paroxetine, some of these drugs act as efflux pump inhibitors in human cells. The influence of these nonantibiotic alternatives occurs in several ways: 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.[4,5]

Fluoxetine is a widely known drug of choice for treating depression; it is an antidepressant drug that belongs to the selective serotonin reuptake inhibitor (SSRI) family. Fluoxetine (Prozac®) was the first SSRI available for clinical use in most countries. Since then, fluoxetine has become one of the most commonly used antidepressants.[6] Studies showed that inhibits lipopolysaccharide (LPS) induced production of tumor necrosis factor-alfa (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO).[6,7] In an in-vitro study, fluoxetine showed significant antibacterial effect and potential antibiotic modulating activity against multiresistant bacteria. Fluoxetine, together with an antibiotic such as gentamicin and erythromycin, used against *Pseudomonas aeruginosa* and *Escherichia coli*, showed synergistic effects. It demonstrates that fluoxetine can selectively modulate the activity of antibiotics for clinical use.[8] This study examinates the antimicrobial effect of fluoxetine compared to the antibiotic imipenem used in the treatment of sepsis. And besides, the possible mechanisms to accelerate and support the treatment by modifying the antibiotic effect by the experimental sepsis model in rats.

2 | MATERIAL AND METHOD

2.1 | Reagent and chemicals

Orthophosphoric acid (H_3PO_4), methanol, ethanol, and ethyl acetate were purchased from Merck Chemical. Acetic acid (CH_3COOH), ninhydrin, aluminum chloride ($AlCl_3$), copper(II) sulfate pentahydrate, quercetin, gallic acid, sodium hydroxide (NaOH), L-Leucine, 2,4,6-Tris(2-pyridyl)-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, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) were purchased from Sigma-Aldrich (Chemie GmbH).

2.2 | Protocols of experimental sepsis model

2.2.1 | Experimental graph

This study was conducted on 40 three-month-old female Wistar Hannover rats weighing 230–250 g. The rats were placed in cages with a 12 h light/dark cycle at room temperature and had free access to regular rodent diet food and tap water.

Minor modifications were made to the cecal ligation and puncture (CLP) method described by Rittirsch et al.[9] 50 mg/kg ketamine hydrochloride and 10 mg/kg xylazine were administered intraperitoneally as anesthesia, and the procedures indicated was performed, respectively. The procedure was terminated with sterile dressing in the operation area.[10,11] 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), see Figure 1. 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 h after the surgical operation and continued for 4 days with an interval of 12 h, and the experiment was terminated after the sixth injection (approximately 72 h after the surgical operation).

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

1. Negative control: Sham operation (surgical incision) to imitate the same after-surgical effects. Rats were only given a Saline solution (0.9 NaCl) every 12 h.
2. Positive control (CLP): CLP to imitate sepsis model. Rats were only given a saline solution (0.9 NaCl) every 12 h.

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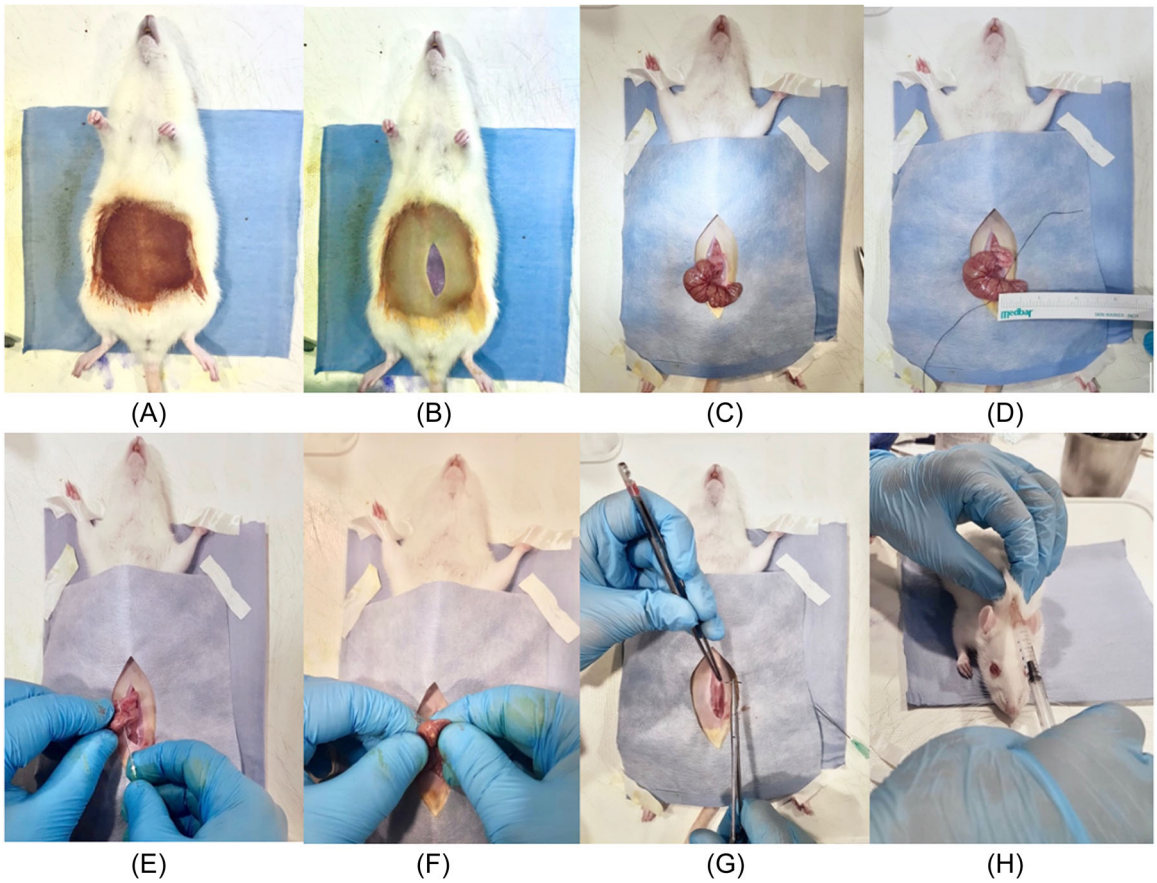

FIGURE 1 CLP sepsis model. (A) The abdomen was shaved and disinfected and (B) the rats were then subjected to laparotomy. The abdominal region was opened from the middle line, (C) the cecum was isolated, (D) after the cecum was filled with stool, it was tied 1 cm below the ileocecal valve, (E, F) a small incision was made, and the flow of intestinal contents into the abdominal cavity was observed, (G) after the cecum had been pierced and the fecal contents contaminated with the peritoneum, the cecum was placed back into the abdomen, and (H) after surgery, the fluid replacement was achieved by injecting 1 ml sterile physiological serum (0.9% NaCl isotonic) subcutaneously. CLP, cecal ligation and puncture.

3. CLP and fluoxetine: Sepsis modeled rats received 5 mg/kg fluoxetine (FLU) drug intraperitoneally every 12 h (10 mg/kg).
4. CLP and imipenem: Sepsis-modeled rats received 60 mg/kg imipenem (IMP) antibiotic intraperitoneally every 12 h (120 mg/kg).
5. Combined treatment (CLP, fluoxetine and imipenem): Sepsis modeled rats received 5 mg/kg FLU and 60 mg/kg IMP (10 mg/kg and 120 mg/kg) intraperitoneally every 12 h.

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

2.3 | Collection of blood and tissue

Seventy-two 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. Blood samples were centrifuged at 3000 rpm for 10 min.

2.4 | Blood and tissue analyzes

2.4.1 | Blood analyzes

Blood culture

Microbiological growth in blood was detected in the blood culture. After the blood, cultures were obtained sterilely from rats according to BD Biosciences protocol. After performing the blood cultures, the data were read with a nonradiometric continuous monitoring system (The BD BACTEC™ FX blood culture system; Bezmialem microbiology department).

Serum and tissue preparation

The liver, kidney, and intestinal blood tests, total antioxidant status (TAS), total oxidant status (TOS), TT, NT, inflammatory markers, and growth factors were measured from the serum samples separated at the end experiment.

Tissues were homogenized with ceramic balls for 1 min with 1× phosphate-buffered saline (PBS) and centrifuged at 12,000*g* for

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30 min at a temperature of 4°C. After centrifugation, the supernatant was taken, and protein concentration was determined with the Bradford assay (Coomassie Plus (Bradford) Protein Assay, Thermo Scientific).

2.4.2 | Routine sepsis tests

Routine sepsis testsThe immunoassay method measured procalcitonin (PCT) levels in serum (Architect i1000sr; Abbott Laboratories). 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). Lactate levels were measured using a blood gas analyzer.

2.4.3 | Inflammation markers

The investigated blood samples were measured by the spectrophotometric method used by commercially purchased enzyme-linked immunosorbent assay (ELISA; Elabscience) to measure MPO, TNF, IL-1β, IL-6, and MCP levels at 450 nm wavelength on a microplate reader (ThermoScientific™ Varioskan™ Flash Multimode Reader).

2.4.4 | Oxidative stress markers

TAS measurement was done according to the method developed by Erel et al.[12] As a standard, ascorbate was used, and results were given as mM ascorbate equivalent/mg protein for tissue.

TOS was performed with another method developed by Erel et al.[13] As a standard, μM H_2O_2 was used, and results were given as μM H_2O_2 equivalent/mg protein for tissue. Oxidative stress index (OSI) was calculated by TOS/TAS.

For the measurement of thiol-disulfide homeostasis, the automated method developed by Erel et al. was used.[14] Total thiol (TT) (—SH + —S—S—) and native thiol (—SH) concentrations in the samples were measured using Ellmann and modified Ellmann reagents.

Mononuclear leukocytes isolation

The comet assay was mononuclear leukocyte isolation performed using Histopaque 1077. Whole heparinized blood was gently layered (1:1) over Histopaque and centrifuged for 25 min at 2100 rpm at room temperature. After centrifuge, the interface band was washed with PBS and collected after 15 min centrifugation at 1890 rpm.

2.4.5 | DNA damage

Microscope slides were coated with 1.0% normal melting point agarose (NMA) agarose in PBS. The microscope slides were covered with a coverslip at +4°C for at least 5 min until the agarose solidified.

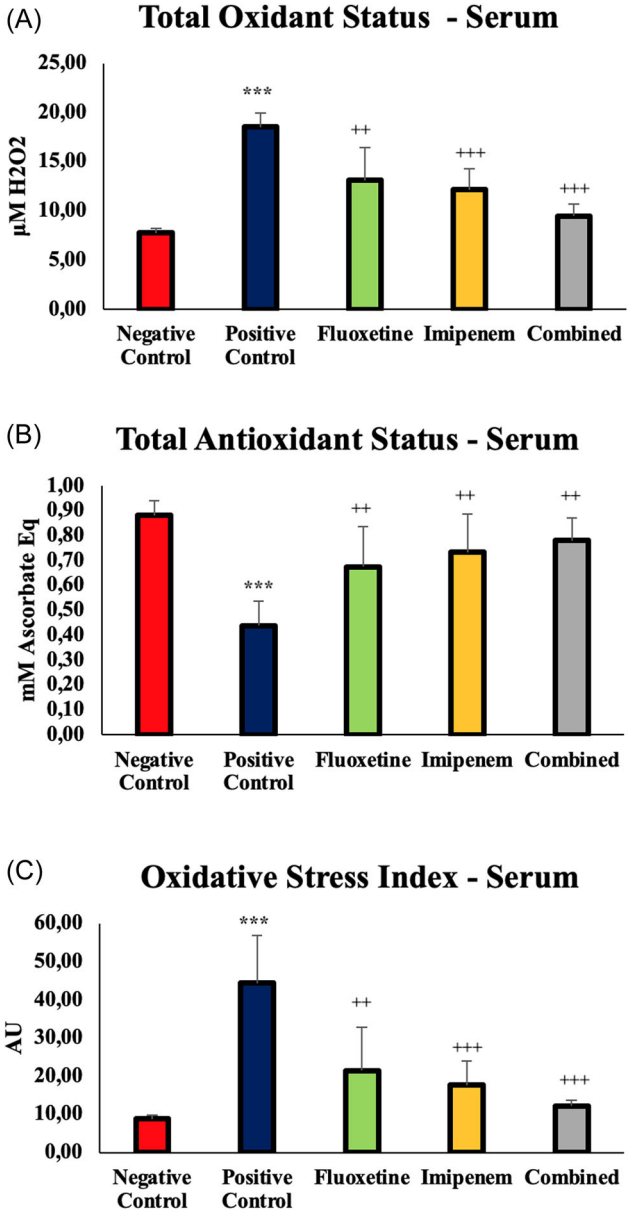

FIGURE 2 Oxidative stress status in serum. (A) Serum total oxidant levels: TOS, (B) serum total antioxidant levels: TAS, (C) serum oxidative stress index: OSI. Data are expressed as the mean ± standard deviation for five to eight animals per group (average ± SD and the *p* values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

After removing the coverslips, the mixture of 85 μl of 0,6% low melting agarose (LMA) and 15 μl isolated mononuclear leukocyte were placed on precoated slides of 1% NMA. The slides were allowed to solidify for 10 min at 4°C. The slides were immersed in the prepared cold lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris-HCl, and pH 10–10.5, 1% Triton X-100) overnight. After the overnight, the slides were incubated in the electrophoresis containing running buffer solution (0.3 mol/L NaOH, 1 mmol/L Na_2 EDTA, pH13) at +4°C in the dark for 40 min. Electrophoresis was implanted in an electrophoresis tank for 35 min at 26 V, 300 mA. The

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slides were stained with ethidium bromide (2 µl/ml H_2O, 70 µl per slide) After electrophoresis and visualized using an epifluorescence-equipped ×200 magnification fluorescence microscope (Leica DM 1000) using Comet assay software IV (Perceptive Instruments).

2.5 | Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 22.0. All experiments were done in triplicate, and the obtained results were expressed as mean value ± standard deviation (mean ± SD). Using the analysis of variance (ANOVA) (one-way ANOVA) was a statistical evaluation performed. The differences with a probability value were accepted statistically ($p < 0.05$).

3 | RESULTS

3.1 | Oxidative stress status

3.1.1 | Oxidative stress status in serum

Oxidative stress is an imbalance between oxidants and antioxidants favoring the oxidants, potentially leading to damage. Therefore, oxidative stress contributes to the sepsis process.

TOS and OSI levels began to decrease by combined treatment of fluoxetine 10 mg/kg and imipenem 120 mg/kg. The oxidative stress biomarker levels of serum in different groups are given in Figure 2A,B. The experimental sepsis model's OSI levels were significantly reduced, mostly after combined treatment with fluoxetine and imipenem ($p < 0.001$).

The TT and NT levels of the treatment groups changed statistically significantly compared to the positive control group. And MPO enzyme decreased significantly by 64.83% in the combined treatment group (fluoxetine and imipenem) compared to the positive control ($p < 0.001$), see Figure 3.

3.1.2 | Oxidative stress status in tissues

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

TOS and OSI levels began to decrease by treatment groups: fluoxetine 10 mg/kg/day, imipenem 120 mg/kg/day, and combined treatment of fluoxetine 10 mg/kg and imipenem 120 mg/kg/day. The changes of oxidative stress biomarker levels of liver, kidney, and ileum tissue in different groups are given in Figure 4. The treatment group fluoxetine decreased to 21.22 ± 19.08 arbitrary units, in the imipenem group decreased to 16.49 ± 11.30 arbitrary units, and the combined group to 7.73 ± 2.52 arbitrary units compared to the

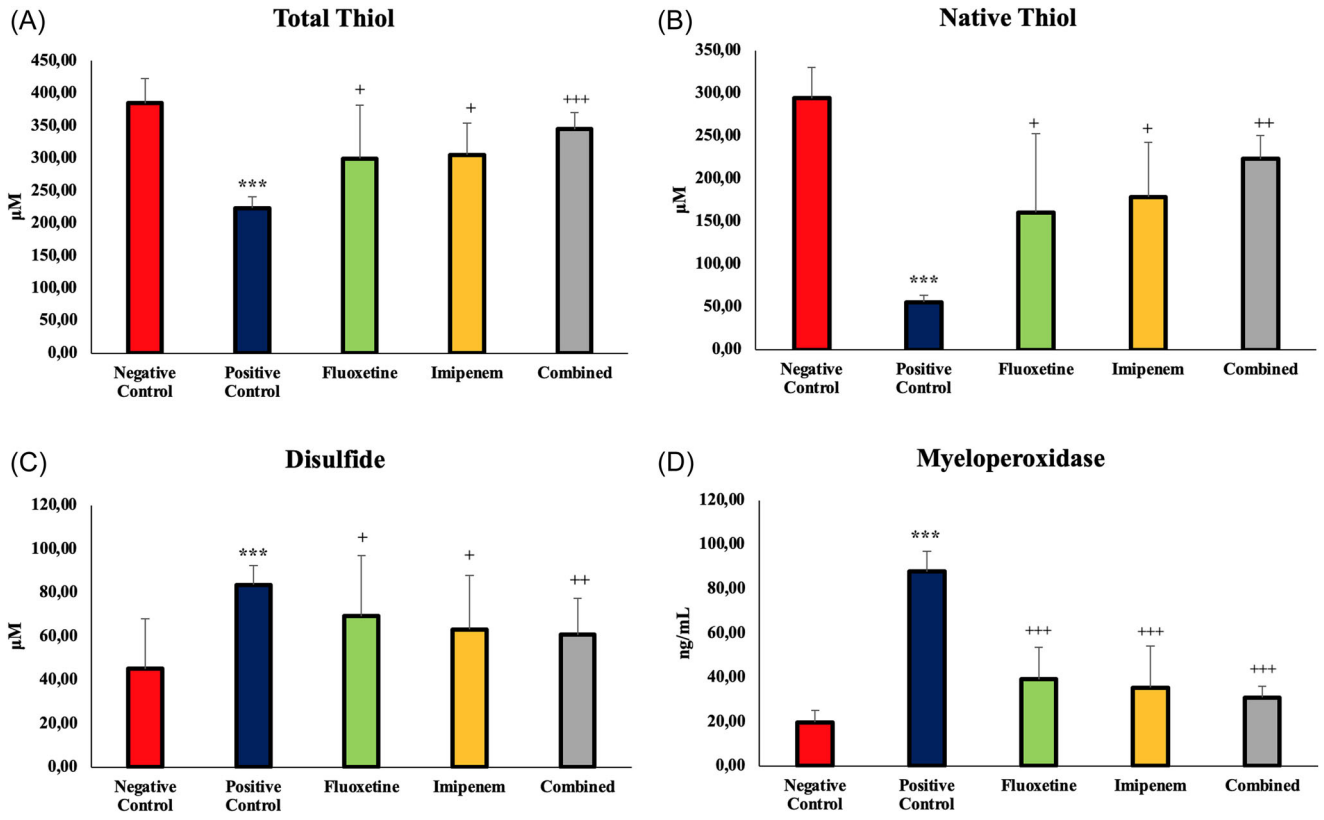

FIGURE 3 Thiol homeostasis in serum. (A) Serum total thiol levels: TT, (B) serum native thiol levels: NT, (C) serum disulfide levels: DIS, (D) and myeloperoxidase (MPO) levels. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD) and the p values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

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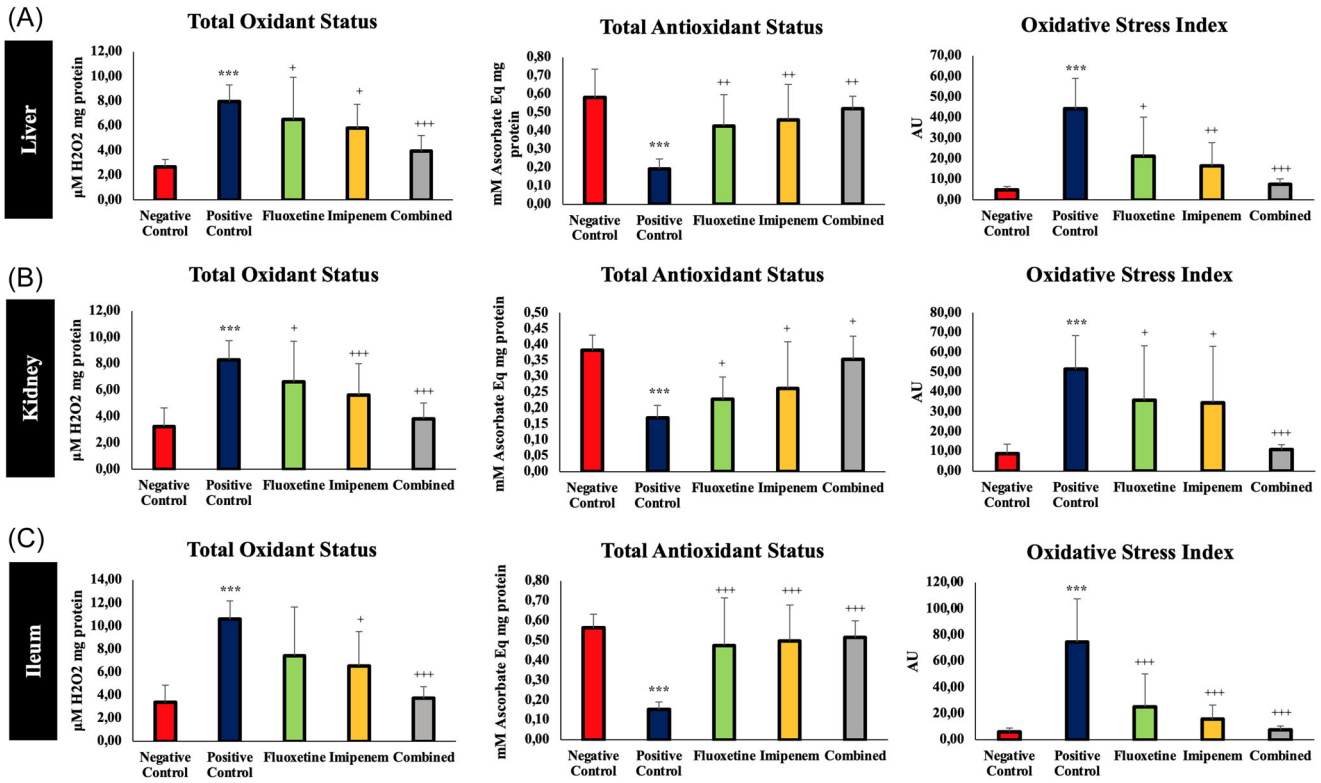

FIGURE 4 The oxidative stress in liver, kidney, and ileum tissues following the experimental sepsis model. (A) Liver total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI). (B) Kidney TOS, TAS, and OSI. (C) Ileum TOS, TAS, and OSI. Also, the changes in all treatment groups are statistically significant compared to the positive control group. Data are expressed as average ± standard deviation (n = 5–8) and the p values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

positive control group ($p < 0.05$) in the liver. In kidney, fluoxetine decreased to 35.62 ± 27.57 arbitrary units, in the imipenem group decreased by to 34.51 ± 28.38 arbitrary units, and combined group to 10.85 ± 2.64 arbitrary units compared to the positive control group ($p < 0.05$). And the changes of oxidative stress biomarker levels of ileum tissue in different groups the treatment group fluoxetine decreased to 25.27 ± 24.86 arbitrary units in the imipenem group decreased to 15.78 ± 10.84 arbitrary units and the combined group to 7.56 ± 3.07 arbitrary units compared to the positive control group ($p < 0.05$).

3.2 | Inflammatory parameters

3.2.1 | Inflammatory parameters in serum

Inflammatory biomarkers levels in all groups can be seen in Figure 5; 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. An experimental sepsis model significantly reduced these cytokine levels after combined treatment with fluoxetine and imipenem ($p < 0.05$).

The most commonly used sepsis biomarkers in the routine lab are PCT and hs-CRP. The hs-CRP and PCT levels in all groups are given in Figure 6. By a combined group of fluoxetine and imipenem, the hs-CRP decreased to 0.27 ± 0.07 ng/ml and PCT to 49.77 ± 13.68 ng/L compared to a positive control (hs-CRP: 7.95 ng/ml and PCT: 389.90 ng/ml).

After treatment with fluoxetine, imipenem, and the combination (fluoxetine and Imipenem), 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 1.

3.2.2 | Inflammatory parameters in tissues

Inflammatory biomarker levels in liver, ileum, and kidney tissue groups can be seen in Figure 7. An experimental sepsis model significantly reduced these cytokine levels after combined treatment with fluoxetine and imipenem ($p < 0.05$).

Pro-inflammatory cytokine IL-1β in the liver decreased to 653.43 ± 80.15 pg/ml mg protein, in the ileum to 859.65 ± 48.20 pg/ml mg protein, and in the kidney to 582.50 ± 63.06 pg/ml mg protein compared to the positive control.

The IL-6 cytokine in the liver decreased to 10.94 ± 1.89 ng/ml mg protein, in the ileum 13.34 ± 3.45 ng/ml mg protein, and in the kidney to 12.78 ± 0.86 ng/ml mg protein compared to the positive control.

And the pro-inflammatory cytokine TNF-α decreased in the liver to 207.16 ± 21.06 ng/L mg protein, in the ileum to 186.33 ± 32.87 ng/L mg

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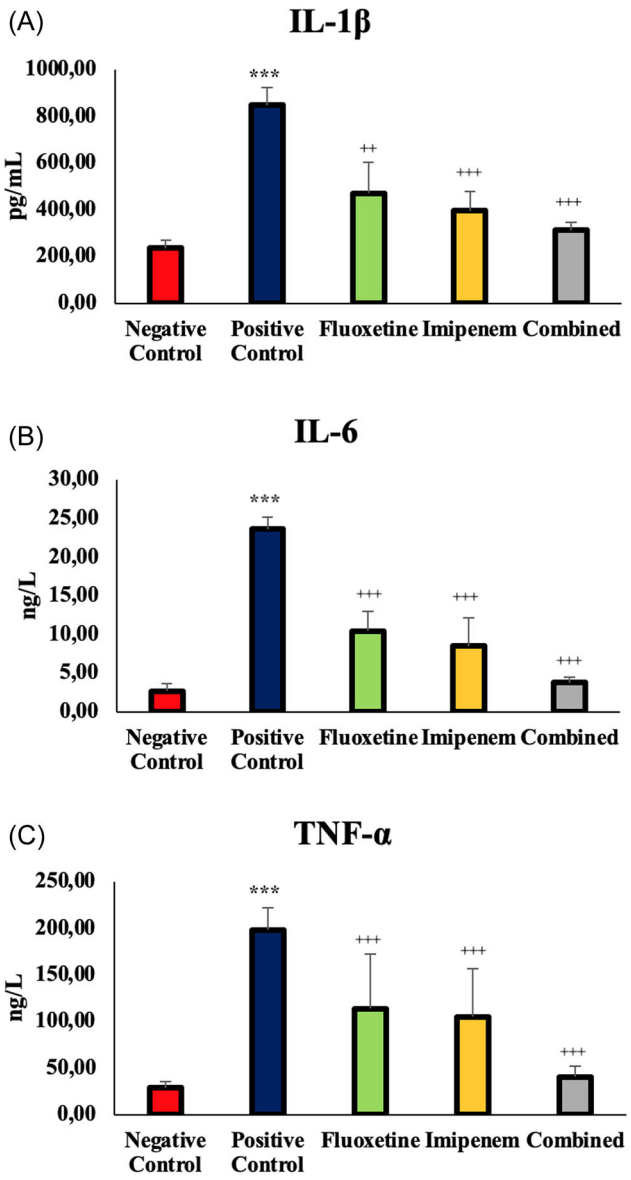

FIGURE 5 Effect of fluoxetine and Imipenem treatment on pro-inflammatory cytokines in the serum of rats. (A) Serum IL-1β level.(B) IL-6 level. (C) TNF-α level. Data are expressed as the average ± standard deviation (SD) for five to eight animals per group (average ± SD) and the *p* values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.0$; 1 $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. IL, interleukin; TNF-α, tumor necrosis factor-alpha.

protein, and in the kidney, to 159.31 ± 45.89 ng/L mg protein in the combined group of fluoxetine and imipenem compared to the positive control.

3.3 | Blood culture

Blood culture was taken to identify the bacteria in the blood—Table 2 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*, *Streptococcusviridans*, and *Staphylococcus haemolyticus* were present. And in the imipenem treatment group, *E. coli*, *Enterococcus faecium*, and *Rodentibacter pneumotropicus* were present.

3.4 | DNA damage

The Comet assay was performed to find the level of DNA damage in the rats of different treatments, as shown in Figure 8. A longer DNA migration smear (comet tail) in positive control can be seen in Figure 8,2b. The DNA damage in the positive group is significantly higher than in the control group.

4 | DISCUSSION

Sepsis is a systemic immune response of the body to an infectious process that leads to organ disorders and the death of millions of people worldwide.[15,16] The lack of cure has led the researchers to a series of nonantibiotic products and medicines. Studies are showing the antimicrobial properties of antidepressants. Similar antimicrobial effects of antidepressants have been identified.[5] SSRIs are the majority of the psychotropic drugs studied in terms of antimicrobial activities.[4] In vitro studies on the antimicrobial and anti-inflammatory effects of drugs such as fluoxetine can alter the pathogenicity of microorganisms or activity on physiology.[4,5] In addition, fluoxetine presents significant potential antibiotic modulating activity against multiresistant bacteria.[8]

This study investigates the antimicrobial effect of fluoxetine compared to the antibiotic imipenem used in the treatment of sepsis and the possible mechanisms to accelerate and support the treatment by modifying the antibiotic effect by the experimental sepsis model in rats. This is the first study of antibiotics toward antidepressants in the sepsis model.

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, Group 4 was treated with imipenem antibiotic 120 mg/kg, and Group 5 was combined treatment with fluoxetine 10 mg/kg and imipenem 120 mg/kg through 4 days. Polymicrobial sepsis was generated by the CLP method.[17] After the treatment, the blood samples and tissues were studied for hs-CRP, PCT, lactate, MPO, IL-1β, IL-6, TNF-α, and MCP-1 were measured using ELISA methods. Different photometric methods studied TT, NT, TOS, and TAS oxidative stress markers.

Blood culture was taken to identify the bacteria in the whole blood. Bacteremia can cause a systemic inflammatory reaction syndrome and lead to severe life-threatening sepsis. No bacteria were present in the negative control group; this was also the intention because no sepsis model was created. In a positive group whereby CLP is used, bacteria *E. coli*, *S. viridans*, and *S.*

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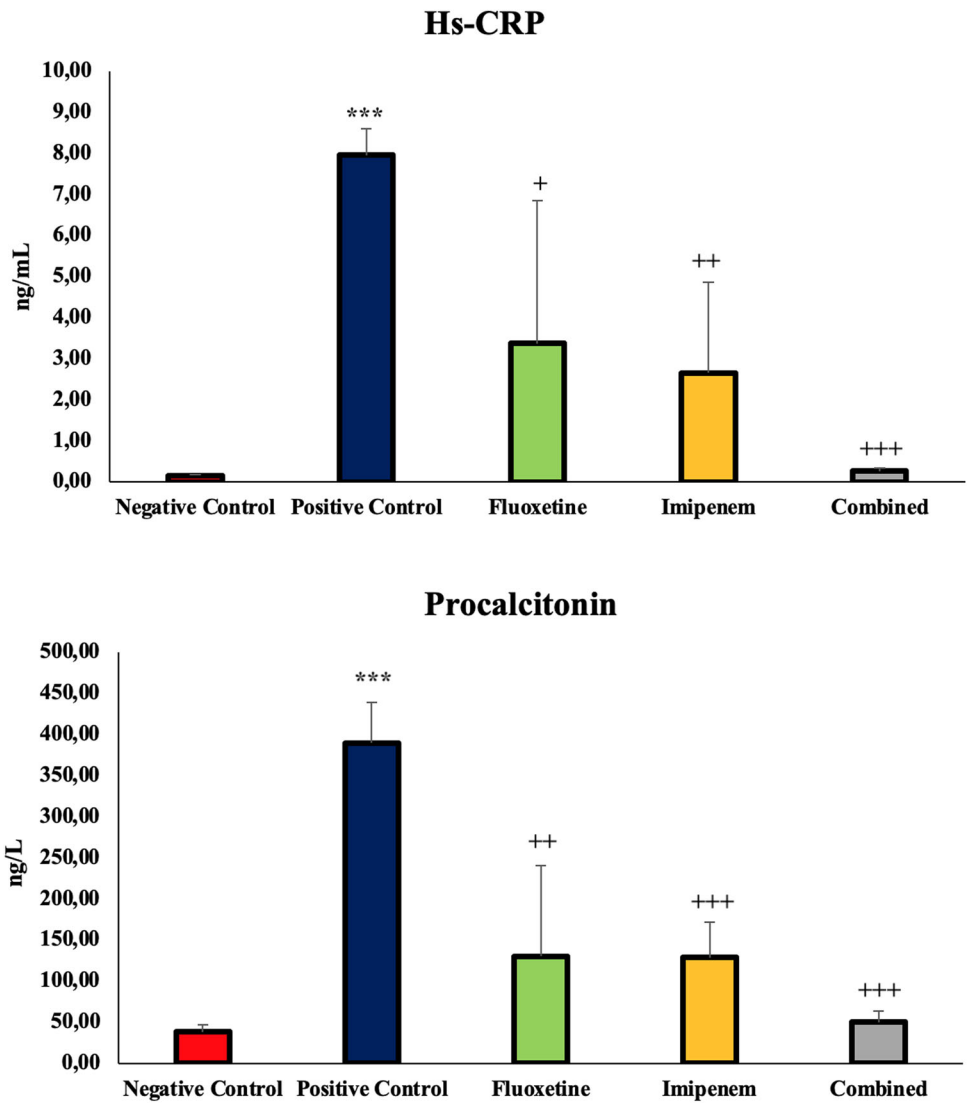

FIGURE 6 hs-CRP and procalcitonin levels of serum in rats. Data are expressed as the mean ± standard deviation for five to eight animals per group (mean ± SD) and the *p* values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.0$; 1 $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. hs-CRP, high-sensitivity C-reactive protein.

TABLE 1 The results lactate and MCP-1 in the serum of the rat

		Negative control (*n* = 7)	Positive control (*n* = 7)	Fluoxetine (10 mg/kg)(*n* = 7)	Imipenem (120 mg/kg)(*n* = 7)	Combined (10 mg/kg + 120 mg/kg)(*n* = 7)
Lactate (ng/µl)	Mean ± 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)	Mean ± SD	202.59 ± 29.56	320.49 ± 93.27***	238.38 ± 45.51^{+}	221.99 ± 48.39^{++}	181.56 ± 35.67^{+++}

Abbreviations: MCP-1,monocyte chemoattractant protein-1; SD, standard deviation.
$^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$; $^{***}p < 0.001$ compared with the positive group (sepsis).

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 and urogenital tracts. These bacteria may have been detected through contamination during the surgical procedure. In the combined group of 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, leading to damage and tissue injury.[18] Therefore, oxidative stress contributes to the

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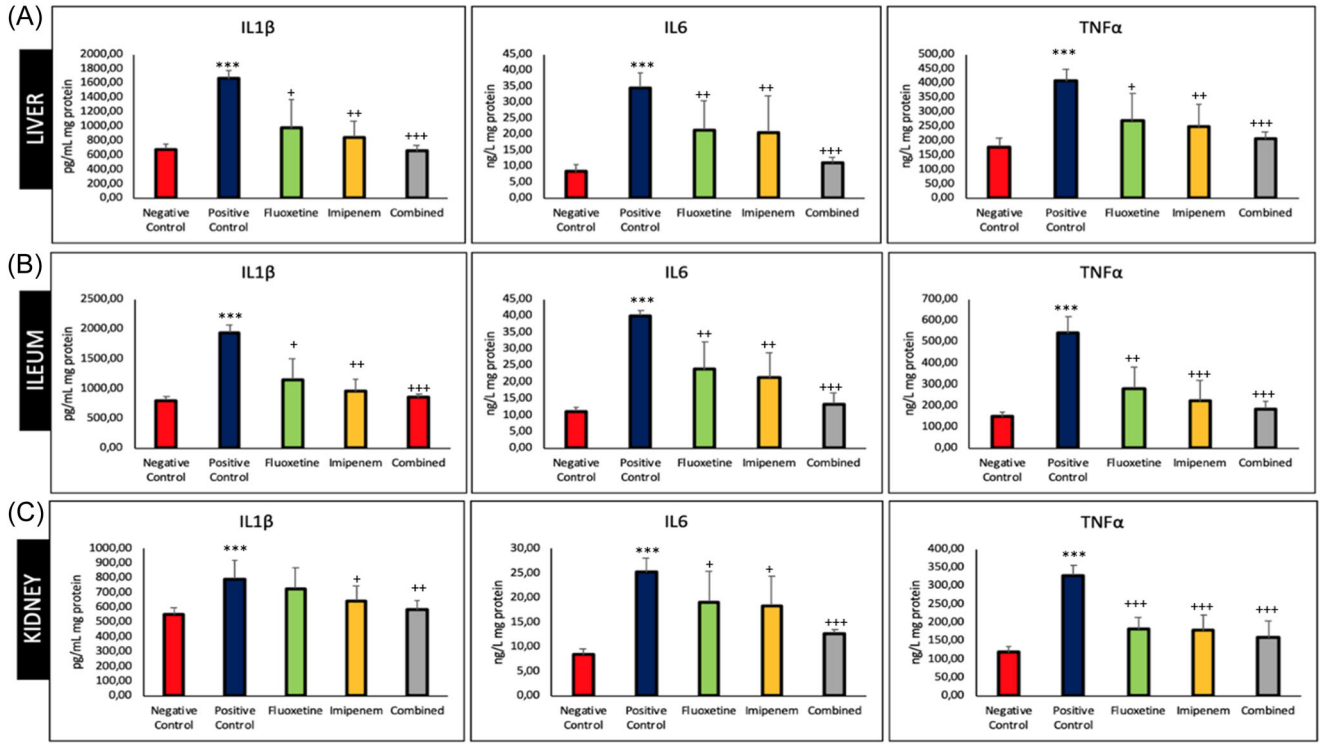

FIGURE 7 Effect of fluoxetine and imipenem treatment on pro-inflammatory cytokines in tissues of rats. (A) Liver tissue IL-1β, IL-6, and TNF-α levels. (B) Ileum tissue IL-1β, IL-6, and TNF-α levels. (C) Kidney tissue IL-1β, IL-6, and TNF-α levels. Data are expressed as the average ± standard deviation for five to eight animals per group (average ± SD) and the *p* values are compared with the positive group. $^{+}p < 0.05$; $^{++}p < 0.0$; 1 $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. IL, interleukin; TNF-α, tumor necrosis factor-alpha.

TABLE 2 Bacteria identification of bloodstream infection in rats

n	Negative control	Positive control	Fluoxetine	Imipenem	Combined
1	Negative	*Escherichia 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*	*Enterococcus faecium*	Negative
5	Negative	*Streptococcus viridans*, *Staphylococcus haemolyticus*	Negative	*Enterococcus faecolis*	Negative
6	Negative	*E. coli*	*E. coli*	Negative	Negative
7	Negative	*E. coli*	Negative	*Rodentibacter pneumotropicus*	Negative

sepsis process. Different photometric methods studied TT, NT, TOS, and TAS oxidative stress markers. The TT and NT levels of the treatment groups changed statistically significantly compared to the positive control group. Our study determined the oxidative stress according to the OSI. The highest OSI level of serum was by positive control. The experimental sepsis model significantly reduced the OSI level by first fluoxetine and then imipenem. But it mainly decreased after combined treatment with fluoxetine and imipenem. In addition, oxidative stress indicators were investigated in the experimental groups' liver, kidney, and ileum to determine the mechanism of liver, kidney, and ileum damage caused by sepsis. In early sepsis, liver dysfunction is frequently seen from altered hepatocellular functions.[19] 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.[20] TOS and OSI levels in the liver began to decrease by all treatment groups: fluoxetine, imipenem, and combined treatment of fluoxetine and imipenem. From the

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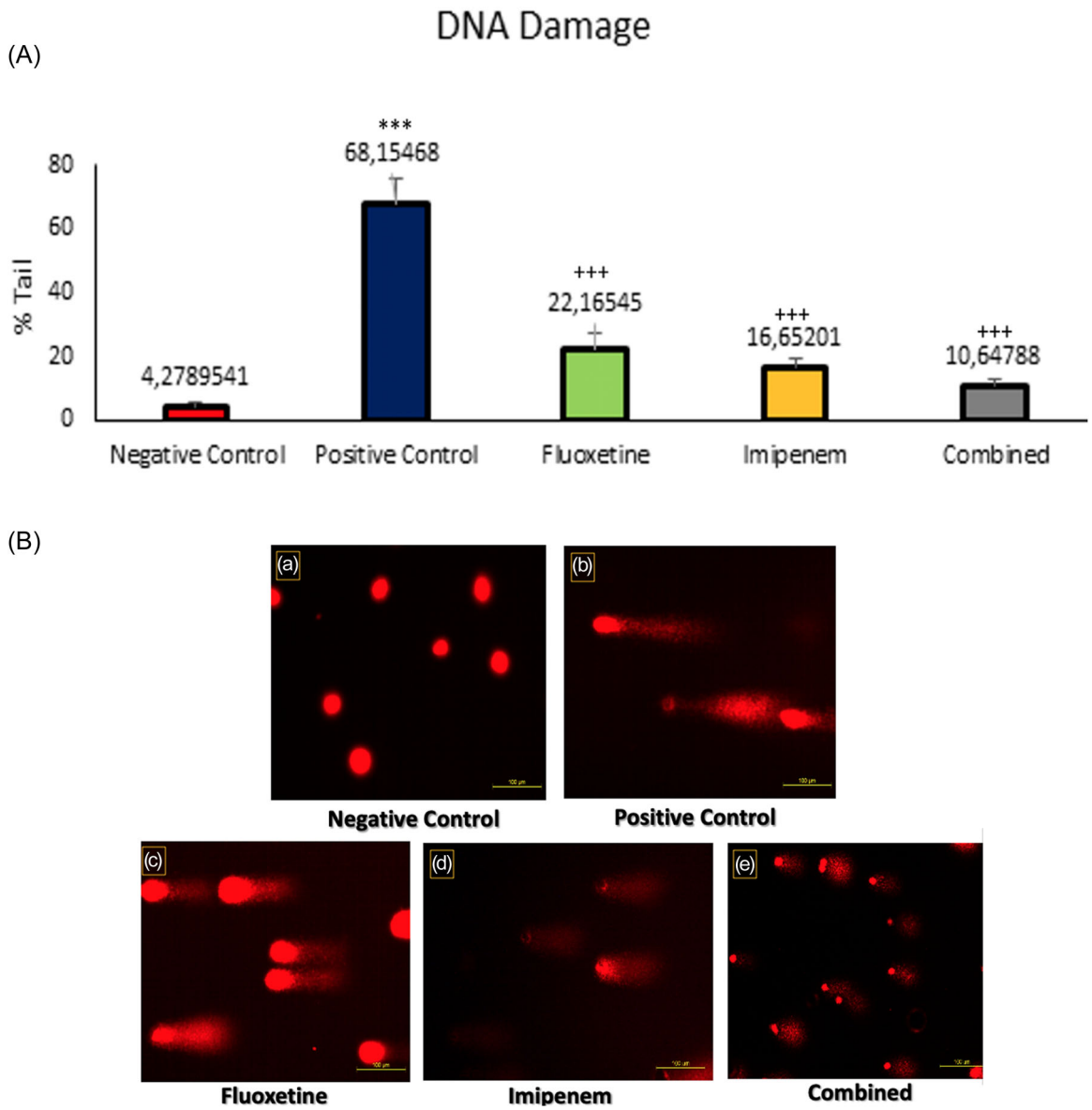

FIGURE 8 Comet Assay analysis of DNA damage in rats versus healthy controls. (A) Comet tail length after treatment; fluoxetine, imipenem, and combination (fluoxetine + iImipenem) groups. The *p* values are compared with the positive group $^{+}p < 0.05$; $^{++}p < 0.0$; 1 $^{+++}p < 0.001$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with the positive group. (B) 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 10 mg/kg/day, (d) treatment group with imipenem 120 mg/kg/day, and (e) Combined group treatment with fluoxetine 10 mg/kg and imipenem 120 mg/kg/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.

results, OSI levels were highest decreased by the combined treatment.

A study found apoptosis and necrosis increase during severe sepsis and septic shock.[21] After isolating 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. The results indicated that in the fluoxetine group, the damage was lowest observed by the combined group 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.[22] A decrease of at least 20% in lactate value is a vital in-patient prognosis.[23] The results show that the serum lactate levels 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.

Fluoxetine inhibits LPS-induced production of TNF-α and IL-6.[7] From the inflammatory response panels results, the positive control group in which sepsis has been created has a high percentage of TNF,

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IL-1β, IL-6, MCP-1, and hs-CRP. This was also the intention because, by sepsis, the damaged tissue cells are recognized by macrophage receptor TLR, leading to the production of pro-inflammatory cytokines TNF, IL-1β and IL-6, and MCP-1. The IL-6 stimulates the liver to produce hs-CRP.[24] The most commonly used sepsis biomarkers in the routine lab are PCT and hs-CRP. The role of hs-CRP during acute inflammation is to bind the phospholipid components of microorganisms and damage host cells. This biomarker is commonly used to screen for early stage of sepsis.[25] From the results of this research, the hs-CRP and PCT in serum were highly decreased by the combined group of fluoxetine and imipenem compared to the positive control.

MPO enzyme was evaluated as an indicator of the inflammatory process. A significant increase was detected in the positive group compared to the negative group in our results. And the mainly decreased percentage was in the combined (fluoxetine and imipenem) group compared to the positive control.

In the first period of inflammation, the cytokines TNF-α, IL-1β, and IL-6 play the leading role, the leukocytes, the primary production source. One of the most exciting studies explaining that TNF-α is responsible for organ injury and death in sepsis was performed by Waage et al. in 1987. The study states that sepsis patients have high TNF-α levels, which is a turning point in understanding sepsis.[26] Many studies have confirmed a close relationship between increased plasma TNF-α levels and increased mortality.[27,28]

Our study 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 efflux pump inhibitor in human cells. The inhibition of these pumps appears to be an attractive strategy when shrinking the number of antibiotics.[4] In addition, fluoxetine inhibits LPS in which is present in gram-negative bacteria, and the production of TNF-α, IL-6, and NO was induced.[6,7] A study found that fluoxetine suppressed the number of cells associated with the inflammation and TNF-α release from monocytes in an animal model of septic shock.[29] Another study showed that the association of fluoxetine with antibiotic gentamicin or erythromycin showed synergistic effects. They demonstrated that fluoxetine increased the effectiveness of the antibiotics.[8] This also corresponded to our results. In our study, the rats were treated with an antibiotic primarily used in sepsis, namely imipenem. This antibiotic belongs to the Carbapenem family and works by disrupting the bacteria's cell wall. Fluoxetine and imipenem drugs can have direct antimicrobial activity and increase an antibiotic's efficiency when given together. [4]

5 | CONCLUSION

As a result of our study, the combined therapy of fluoxetine and imipenem has been shown to have an anti-inflammatory and antioxidant effect due to its synergistic effects in the in-vivo sepsis model. Considering the in-vivo results in our study, it was thought that fluoxetine might 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 oral or intravenous formulations of fluoxetine can be helpful for treatment.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available on request due to privacy or other restrictions.

ORCID

Ahsen Cakir http://orcid.org/0000-0002-2121-2198
Kubra Bozali http://orcid.org/0000-0003-2416-0773
Mert Celikten http://orcid.org/0000-0002-8215-3638
Eray Metin Guler http://orcid.org/0000-0003-4351-1719
Ebru Sahan http://orcid.org/0000-0002-1287-8166
Bulent Durdu http://orcid.org/0000-0002-0244-4006
Bilge Sumbul http://orcid.org/0000-0002-8768-3777
Abdurrahim Kocyigit http://orcid.org/0000-0003-2335-412X

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