http://jbcp.shahed.ac.ir 10.22070/JBCP.2024.19793.1187

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol. 13, No. 1, February 2025

A Semiannual Publication of
Shahed University Press

The pathophysiological effects of ciprofloxacin on biochemical and histological changes in liver, kidney, and hippocampus pyramidal cells in male Wistar rats

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Abstract

Background and Objective: Ciprofloxacin is effective against a wide range of bacterial illnesses. This investigation evaluated the pathophysiological effect of ciprofloxacin on liver, kidney, and hippocampus pyramidal cells in male Wistar rats.

Materials and Methods: Forty adult Wistar rats were randomly assigned to four groups: control (Saline 0.5 ml, orally, 30 days) and ciprofloxacin (25, 50, 100 mg/kg, orally, 30 days). Rats were euthanized when the treatment was completed. Blood samples were collected for chemical analysis, while liver, kidney, and brain samples were excised for histological examination.

Results: Compared to the control group, ciprofloxacin-treated groups exhibited significant increases in MDA, liver enzymes, urea, and creatinine levels, whereas SOD, GSH, and CAT enzyme levels were significantly reduced. Ciprofloxacin treatment induced severe vacuolation, marked hepatocyte degeneration, dilated sinusoids, congested central veins, severe hepatic hemorrhage, glomerular atrophy, as well as several changes, including dilation of renal convoluted tubules, degenerated epithelial cells, and luminal dilation. In the ciprofloxacin-treated groups, the number of healthy neurons in CA1, CA2, and CA3 hippocampus areas decreased dose-dependently as opposed to the control group.

Conclusion: By increasing oxidative stress, ciprofloxacin was found to elevate serum levels of liver enzymes, urea, and creatinine. Moreover, it induced histopathological changes in the liver and kidney and decreased the number of healthy neurons in hippocampal pyramidal cells compared with the control group.

Keywords: Ciprofloxacin, Hippocampus, Kidney, Liver, Oxidative stress

1. Introduction

The liver is a complex organ with three main functions: metabolic, storage, and secretory. Hepatocytes synthesize circulating proteins and are responsible for establishing bile acid-dependent cholestatic flow, detoxifying foreign bodies, and regulating intermediary metabolism (1). Some factors cause cell death in the liver, including immune factors, endogenous factors such as toxic bile acids and free fatty acids, and exogenous factors such as viruses, alcohol, substance abuse, and certain medications (2).

The kidney controls the volume of different body fluids, the osmolality of fluids, and acid-base balance, regulates the concentration of different electrolytes, and removes toxins. In acute renal failure, cell death may be a direct consequence of exposure to noxious stimuli. Many renal lesions mainly affect the tubular epithelial cells, which are metabolically very active (3).

The hippocampus is part of the limbic system. This part of the brain has a folded structure and is located bilaterally deep in the temporal lobe. The basic

Received: 16 November 2024, **Revised:** 14 December 2024, **Accepted:** 21 December 2024
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structure of the hippocampus consists of three areas: cornu ammonis, dentate gyrus, and subiculum. The hippocampus is divided into three areas, namely, CA1, CA2, and CA3. Different areas of the hippocampus are composed of different layers, with pyramidal cells forming the primary cellular layer in these regions (4). Substantial data indicate that in humans and animals, the hippocampus is critical for creating new memories, and studies have shown that any damage to the hippocampus causes impairment in recalling memories (5).

Despite the beneficial effects of antibiotics, these compounds have the potential to alter the body's natural microbial composition. Disrupting the human body's natural flora might also increase the likelihood of secondary infections by giving drug-resistant germs a fertile breeding ground (5). The pharmacological or toxicological qualities of the antibiotic, hypersensitivity, or allergic responses may all contribute to side effects. Adverse effects may range from mild symptoms, such as fever and nausea, to severe reactions, including photodermatitis and anaphylaxis (6).

Another harmful effect of antibiotics is the generation of reactive oxygen species (ROS). When these ROS are produced at levels exceeding the cell's defensive mechanisms or are allowed to accumulate for an extended period, they can cause cell dysfunction and death. Cross-linking of thiol groups in proteins and induction of lipid peroxidation are additional ROS-associated effects (5). Oxidative stress plays a pivotal role in the death of liver and kidney cells. ROS can trigger cellular apoptosis or necrosis by functioning as signaling molecules in cell death pathways or by directly causing oxidative damage to cellular macromolecules, such as DNA, proteins, and lipids (7).

Ciprofloxacin, a fluoroquinolone antibiotic, demonstrates efficacy against a wide range of bacterial infections, including those of the skeletal system (bone and joint), gastrointestinal tract, respiratory tract, skin and soft tissues, and urinary tract. Additionally, it is effective in treating systemic infections like typhoid fever (8). However, its administration is linked with certain side effects. The Food and Drug Administration has reported adverse events, including toxic epidermal necrolysis, Stevens-Johnson syndrome, hypotension, allergic pneumonitis, bone marrow suppression, hepatitis or liver failure, and photosensitivity (9).

The current study investigated the effect of different ciprofloxacin doses on the functioning and histological structure of the liver, kidney, and hippocampus pyramidal cells in male Wistar rats.

2. Materials and Methods

2.1. Animals

Male Wistar rats (aged 90 days, weighing 250 ± 10 g) were procured from the Pasteur Institute (Tehran, Iran). They were housed in polypropylene cages under appropriate laboratory conditions (23±2ºC, 40٪ - 60 ٪humidity, ventilation, and a 12-hour light/dark cycle). Each cage housed a maximum of five rats. Rats were fed on the standard chow and drinking water ad libitum throughout the experiment. A 15-day acclimatization period preceded the experimental assay. All procedures were approved by the local ethical committee (Research and Ethics Committee of the School of Biology, University of Arak; IR.ARAKMU.REC.1402.068).

2.2. Experimental design

Male Wistar rats were allocated to 4 groups (n=10): a control group (normal saline 0.5 ml, orally) and three ciprofloxacin (SIGMA, USA) groups (25, 50, 100 mg/kg, orally). Treatments were performed every day for 30 days (10).

At the end of the treatment, the animals in each group were randomly divided into two groups. Animals in the first group were euthanized for blood serum measurement and histological examinations of the liver and kidney. Animals in the other group were used for brain perfusion and analysis of hippocampal pyramidal cell number.

2.3. Biochemical analysis

Blood samples were collected from the heart in non-heparinized tubes. They were centrifuged using a refrigerated centrifuge (Universal, made in Germany) at 4ºC (13,300 rpm for 10 min), and the supernatant was frozen at -20◦C in aliquots for subsequent biochemical assays. In order to evaluate liver enzymes and kidney function, serum levels of aspartate aminotransferase (AST) (BioSystems kits, USA), alanine transaminase (ALT) (Spectrum kit, Egypt), alkaline phosphatase (ALP) (BioSystems kits, USA), serum urea (BioSystems kits, USA) and creatinine (BioSystems kits, USA) were measured in all groups using commercially available kits according to the manufacturer's instructions.

2.4. Antioxidant assessments

Blood serum was analyzed to measure antioxidant capacity across experimental groups. Malondialdehyde (MDA) levels were measured using the thiobarbituric acid (TBA) method. In this method, aldehydes react with thiobarbiturates to form pink complexes. These complexes can be measured by spectrophotometry at 535 nm wavelength and expressed as µmol/ml (11). The level of superoxide dismutase (SOD) enzyme activity was measured using pyrogallol. Pyrogallol oxidizes spontaneously in

aqueous and alkaline environments. The enzyme superoxide dismutase prevents the spontaneous oxidation of pyrogallol. Enzyme activity is measured at a wavelength of 420 nm and is expressed as U/ml (12). The activity of the catalase enzyme was measured using potassium phosphate buffer based on the spectrophotometric measurement of H2O2 decomposition at 240 nm (13), and the results were expressed as U/ml. Serum glutathione levels were measured using the GSH assay kit (Bethesda, MD, USA) according to the manufacturer's instructions. The results were expressed as µmol/ml.

2.5. Histological studies

Half of the animals in each group underwent tissue collection. Liver and kidney tissues were excised after blood collection, rinsed with saline, and fixed in 10% formalin. Subsequent procedures included tissue processing, paraffin embedding, microtomy (Leitz 1512), and hematoxylin-eosin staining. Stained slides were examined using a light microscope (BX40, Olympus, New York, USA) attached to a camera (Olympus, DP12) and quantitatively analyzed with ImageJ software (14).

In order to collect brain samples, rats were anesthetized with 3.5% chloral hydrate (35 mg/100 g intraperitoneally). They were subsequently perfused with phosphate buffer (PBS, 0.1 M, pH 7.4), followed by 4% paraformaldehyde in pre-cooled physiological saline through the left ventricle. Once removed, the brains were immersed in the same solution (24 or 48 h), processed, and embedded in paraffin. Coronal sections (7 µm thick) of the dorsal hippocampus were stained with hematoxylin and eosin. These sections were obtained from the region between -3.8 and -4.30 mm posterior to bregma, as delineated by the hippocampal structure in the Paxinos Atlas (15). Four sections with equal intervals were selected for neuron count, and the average of these counts was used to estimate the total neuron number in the sample (16).

2.6. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed using one-way analysis of variance (ANOVA) and post hoc Tukey test. Statistical significance was set at the alpha level of 0.05. All statistical analyses were performed using SPSS.

3. Results

3.1. Comparison of liver enzymes between experimental groups

The current study's results showed significant differences between all study groups in liver enzymes (P>0.05). In ciprofloxacin-treated groups (25-100 mg/kg), liver enzyme levels increased dose-dependently compared to the control group (Table 1).

Table 1. Effect of different doses of Ciprofloxacin On liver enzymes

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control Saline (0.5 ml)	12.26± 0.068 [d]	15.353 ± 0.026 [d]	12.394 ± 0.015 [d]
Ciprofloxacin (25 mg/kg)	22.069 ± 0.47 [c]	22.194 ± 0.208 [c]	20.413 ± 0.25 [c]
Ciprofloxacin (50 mg/kg)	33.284 ± 0.35 [b]	31.276 ± 0.19 [b]	30.022 ± 0.14 [b]
Ciprofloxacin (100 mg/kg)	46.115 ± 0.49 [a]	42.456 ± 0.79 [a]	40.643 ± 0.42 [a]

The results are represented as mean ± SEM. Different small letters in each column shows a significant difference (P<0.05) between groups.

3.2. Comparison of kidney function between experimental groups

There were significant differences between all study groups in kidney function (P<0.05). In ciprofloxacin-treated groups (25-100 mg/kg), serum urea and creatinine levels increased dose-dependently compared to the control group (Table 2).

Table 2. Effect of different doses of Ciprofloxacin on Kidney function

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Control Saline (0.5 ml)	33.463 ± 0.029 [d]	0.6414 ± 0.007 [d]
Ciprofloxacin (25 mg/kg)	38.464 ± 0.44 [c]	0.7983 ± 0.009 [c]
Ciprofloxacin (50 mg/kg)	47.978 ± 0.28 [b]	2.3158 ± 0.091 [b]
Ciprofloxacin (100 mg/kg)	63.409 ± 1.11 [a]	3.5541 ± 0.030 [a]

The results are represented as mean ± SEM. Different small letters in each column shows a significant difference (P<0.05) between groups.

3.3. Comparison of serum antioxidant levels between experimental groups

There were significant differences between experimental groups regarding MDA, SOD, GSH, and CAT ($P<0.05$). MDA levels increased while SOD, GSH, and CAT enzyme levels decreased dose-dependently in ciprofloxacin-treated groups (25-100 mg/kg), as compared with the control group (Table 3).

Table 3. Effect of different doses of Ciprofloxacin on Oxidative stress, and antioxidants

Groups	MDA (µmole/ml)	SOD (U/ml)	GSH (µmole/ml)	CAT (U/ml)
Control Saline (0.5 ml)	1.608 ± 0.003 [d]	2.179 ± 0.004 [a]	2.505 ± 0.007 [a]	0.637 ± 0.003 [a]
Ciprofloxacin (25 mg/kg)	2.997 ± 0.007 [c]	1.85 ± 0.004 [b]	2.111 ± 0.008 [b]	0.483 ± 0.02 [b]
Ciprofloxacin (50 mg/kg)	4.211 ± 0.027 [b]	1.415 ± 0.013 [c]	1.794 ± 0.021 [c]	0.373 ± 0.007 c
Ciprofloxacin (100 mg/kg)	6.332 ± 0.063 [a]	0.898 ± 0.014 [d]	1.196 ± 0.020 [d]	0.244 ± 0.006 [d]

The results are represented as mean ± SEM. Different small letters in each column shows a significant difference ($P<0.05$) between groups.

3.4. Liver histological study

Histological changes in the control group exhibited normal liver tissue. Hepatocytes were arranged in a hexagonal radial shape around the central vein, and the bile duct appeared normal (Fig. 1A and B). The ciprofloxacin-treated group (25 mg/kg) displayed histological evidence of hepatic injury, including focal areas of mild hepatocellular necrosis. Additionally, the normal radial arrangement of hepatocytes around central veins was disrupted, and sinusoidal dilation was observed (Fig. 1C and D). Severe degeneration of hepatocytes with a loss of radial arrangement was observed in the 50 mg/kg ciprofloxacin-treated group. In addition, there were sinusoidal dilatation and profiler Kupffer cells with central venous congestion (Fig. 1E and F). In the 100 mg/kg ciprofloxacin-treated group, there was a severe vacuolation of hepatocytes and dilation of sinusoids. Moreover, there was marked hepatocyte degeneration, loss of cord integrity, and severe hemorrhagic congestion (Fig. 1G and H).

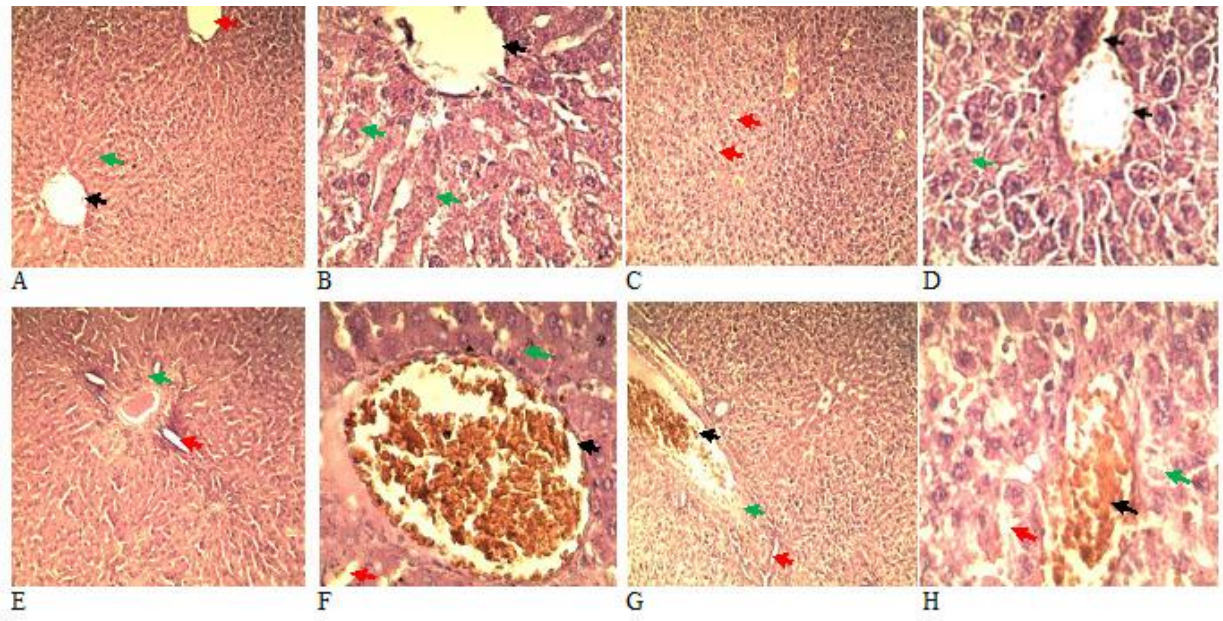

Figure 1. Effect of different doses of Ciprofloxacin on histological changes in the liver tissue. A and B: in the control group, hepatocytes hexagonal radial shape (green arrow) around the central vein (black arrow), and the normal bile duct (red arrow). C and D: in the ciprofloxacin-treated group (25 mg/kg) disturbance in the radial arrangement of liver cells (green arrow), mild hepatocellular necrosis (red arrow), and sinusoidal dilation (black arrow). E and F: in the 50 mg/kg ciprofloxacin-treated group, Severe degeneration of hepatocytes (green arrow), sinusoidal dilatation (red arrow), and central venous congestion (black arrow). G and H: in the 100 mg/kg ciprofloxacin-treated group, severe dilation of sinusoids (red arrow), marked hepatocyte degeneration (green arrow), and severe hemorrhagic congestion (black arrow), (100, 400X, H&E).

3.5. Kidney histological study

Histological sections from the control group revealed normal glomerular proliferation lined by endothelial cells and normal convoluted renal tubules lined by epithelial cells (Fig. 2A and B). Histological examination of the 25 mg/kg ciprofloxacin-treated group revealed mild glomerular atrophy and renal convoluted tubular dilation with epithelial cell degeneration (Fig. 2C and D). The 50 mg/kg ciprofloxacin-treated group showed marked atrophy of glomeruli with degeneration of epithelial cells in convoluted tubules as well as bleeding in kidney tissues (Fig. 2E and F). In the 100 mg/kg ciprofloxacin-treated group, renal histopathology revealed complete glomerular atrophy and cystic dilation of convoluted tubules, accompanied by epithelial cell necrosis and shedding (Figure 2G and H).

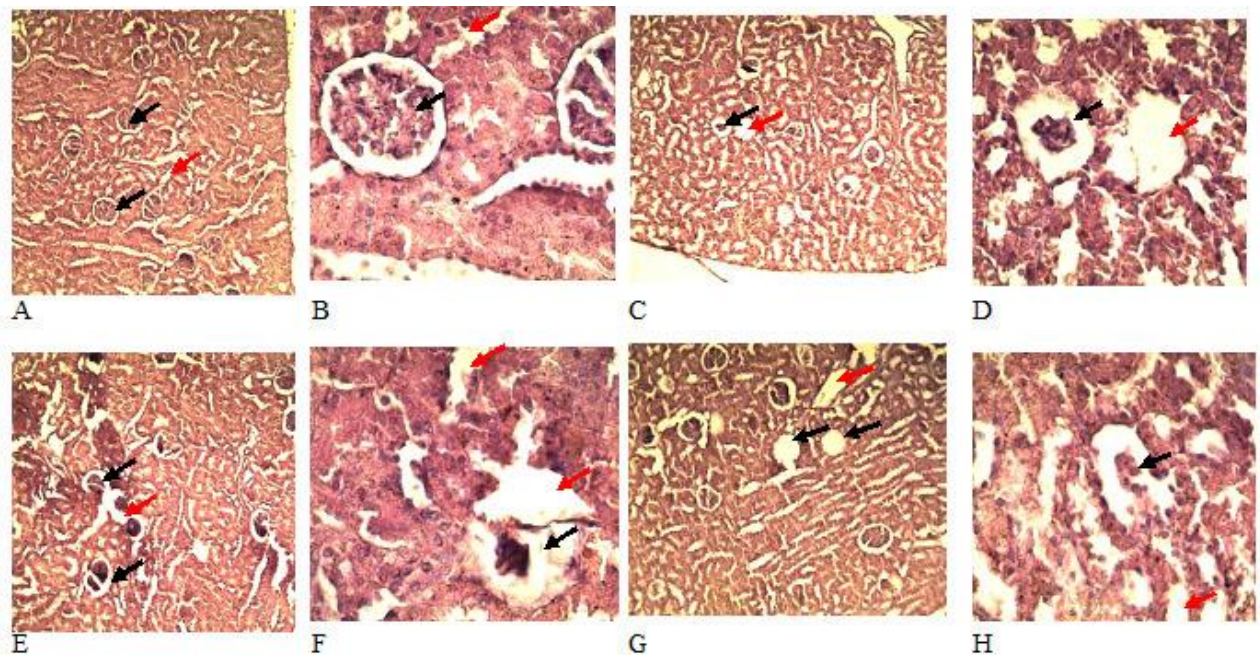

Figure 2. Effect of different doses of ciprofloxacin on histological changes in the kidney. A and B: in the control group, normal glomeruli (black arrow), and normal renal convoluted tubules (Red arrow). C and D: the group treated with ciprofloxacin 25 mg/kg, mild glomerular atrophy (black arrow), and renal convoluted tubular dilation (Red arrow). E and F: the group treated with ciprofloxacin 50 mg/kg, severe necrosis and degeneration in glomeruli (black arrow), and renal convoluted tubular dilation (Red arrow). G and H: The group treated with ciprofloxacin 100 mg/kg, complete glomerular atrophy (black arrow), and dilation in tubules (Red arrow), (100, 400X, H&E).

3.6. Histological study in the hippocampus CA1, CA2, and CA3 regions

The histological section from the control group revealed that the pyramidal cells in the hippocampus's CA1, CA2, and CA3 areas had typical characteristics. They could be seen as circles with a round nucleus. The density of these neurons is high in these areas. In ciprofloxacin-treated groups (25-100 mg/kg), some cells were degenerated or wrinkled without an apparent nucleus, and the color was primarily diffused in the cell. In these groups, the number of healthy neurons in the hippocampus's CA1, CA2, and CA3 regions was reduced dose-dependently compared with the control group (Figure 3).

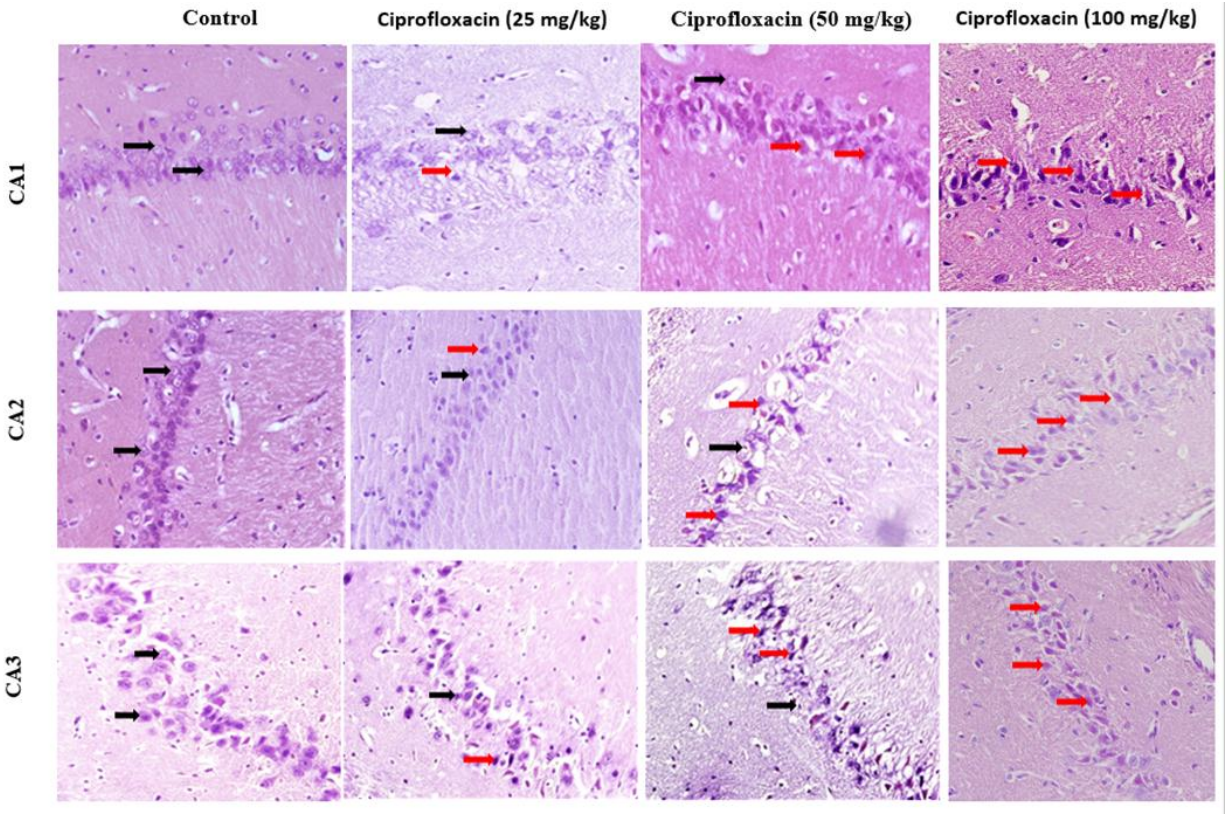

Figure 3. Photomicrographs of typical coronal sections through the CA1, CA2, and CA3 pyramidal neurons of the hippocampus showing Hematoxylin & Eosin in the control group and groups treated with different concentrations of ciprofloxacin. Black arrows show intact pyramidal cells, and red arrows show degenerating pyramidal cells ×400.

4. Discussion

The results of the current study showed that MDA levels increased while SOD, GSH, and CAT enzyme levels decreased dose-dependently in ciprofloxacin-treated groups compared with the control group.

Ciprofloxacin increases ROS production in various cell types. The capacity of ciprofloxacin to generate free radicals may provide mechanistic insight into its side effects (17). ROS, such as superoxide radical anion, hydrogen peroxide, and hydroxyl radicals, cause injury to macromolecules, tissues, and organs via lipid peroxidation, protein modification, and DNA strand breaks (18). Research has shown that the administration of ciprofloxacin in rats increases glutathione and MDA levels, thereby reducing oxidative equilibrium. MDA is a consequence of lipid peroxidation and a key factor in determining free radical formation levels (19). ROS production by fluoroquinolones has been linked to cellular injury in the liver and kidneys (20). Research findings indicate that ciprofloxacin changes the redox state of glutathione in liver and brain tissues, increases MDA and NO levels, and decreases SOD enzyme activity in the brains of ciprofloxacin-treated rats (21).

This study showed that in ciprofloxacin-treated groups, compared to the control group, there was a significant increase in liver enzymes along with histological changes, such as severe vacuolation and marked degeneration of hepatocytes, dilation of sinusoids, and severe hemorrhage congestion.

Chemicals, toxins, viruses, and parasites can cause significant harm to the liver (22). Excessive use of antibiotics destroys the liver tissue by harming hepatocytes. The most prevalent adverse effects associated with antibiotic misuse are antibiotic sensitivity, renal failure, or severe liver damage (23). Ciprofloxacin is a very popular antibiotic used for therapeutic prospects. Ciprofloxacin-induced acute hepatotoxicity is linked with increased levels of ALT, AST, and ALP in most ciprofloxacin-treated rats and may cause liver failure (24, 25). Ciprofloxacin significantly decreases liver protein levels. This reduction may result from increased oxidative stress, leading to nucleic acid depletion, DNA damage, and subsequent mitochondrial dysfunction and loss (26). Researchers have shown that ciprofloxacin treatment significantly increases serum total cholesterol, AST, ALT, and ALP concentration and decreases serum total protein and globulin (27). This can cause dyslipidemia, hepato-reno dysfunction, increased oxidative stress (28), liver failure, hepatitis, cholestatic jaundice, acute liver injury, and histopathological changes (29). Data demonstrate that hepatic AOPPs are substantially elevated in rats treated with fluoroquinolones. AOPPs are cross-linked

protein products containing tyrosine and are dependable markers for measuring the degree of oxidant-mediated protein degradation. Oxidized proteins can accumulate and eventually form cytotoxic protein aggregates, which are significant pathogenic contributors to cellular damage (23).

The results of the current study showed that compared to the control group, the ciprofloxacin-treated groups exhibited a dose-dependent increase in serum urea and creatinine levels. They also underwent several changes in their kidneys, including atrophy of glomeruli with dilation of convoluted tubules and degeneration of epithelial cells.

Some researchers have reported that ciprofloxacin in therapeutic concentration has no side effects, while chronic use can cause histopathological abnormalities as well as damage to various organs, including kidneys (10, 30). Studies have reported that ciprofloxacin increases serum lactate dehydrogenase and creatinine levels, among other adverse effects, resulting in reduced kidney function (28). Ciprofloxacin induces oxidative damage and inflammation in addition to severe nephrotoxicity, manifesting as histological abnormalities (31). The crystallization of ciprofloxacin with magnesium and proteins results in intrarenal obstruction and inflammatory changes in the tubular walls, leading to acute renal failure (32). Rat kidneys treated with ciprofloxacin exhibited renal tubule dilatation and vacuolation. The kidneys' prominent function in quinolone metabolism and excretion may account for the DNA-damaging effect of quinolones in kidneys (23, 33).

In the present study, ciprofloxacin treatment resulted in degeneration of pyramidal cells. These neurons appeared wrinkled, lacked a discernible nucleus, and exhibited diffuse cytoplasmic staining. Additionally, compared to the control group, a dose-dependent reduction in the number of healthy neurons was observed in the hippocampus's CA1, CA2, and CA3 regions.

Treatment with antibiotics leads to a lower number and proliferation of basal cells in the hippocampus, disturbance in the permeability of the blood-brain barrier, changes in neural activity, reduced synaptogenesis, and neuronal destruction (5). The intestinal bacterial flora is closely linked with the proliferation of nerve cells and the activation of glial cells through the brain-gut axis (34). Antibiotics increase intestinal permeability and the migration of some bacteria to the brain, leading to increases in beta amyloids, immune responses, and pro-inflammatory cytokines in the brain (35). Inflammatory factors lead to cell death by increasing the permeability of neurons, activation of astrocytes and microglia cells, damage in the axon of nerve cells, and activation of inflammatory signals in memory-related areas (36). It has been found that antibiotics can increase ROS by impairing mitochondrial function. High accumulation of ROS can disrupt neurons' function and reduce the cell's energy production rate and autophagy, resulting in waste accumulation in the cell and, eventually, cell death (5).

Conclusion

The results revealed that treatment with ciprofloxacin (25-100 mg/kg/day) increased liver enzyme, urea, and creatinine levels in serum dose-dependently and had significant histological changes in the liver and kidney. This treatment also significantly reduced the number of healthy neurons in various hippocampal regions and increased oxidative stress factors in blood serum.

Competing interests

None of the authors have a conflict of interest related to this work.

Authors' contributions

ND: Formal analysis- wrote the main manuscript text
AJ: Investigation. ND and AJ: prepared figures. All authors reviewed the manuscript.

Acknowledgements

The authors would like to thank the Arak University for valuable cooperation.

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