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RESEARCH ARTICLE

Assessment of Ciprofloxacin Effects on some Chicks' Organs: A Comprehensive Biochemical and Histological Study

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ABSTRACT

The aim is to evaluate ciprofloxacin toxicity in the liver and kidneys by assessing their functioning, histological alterations, and GFAP expression in chicks. In the acute trial, the chicks were divided into 3 groups of six. The 1st was the control. The 2nd and 3rd received injections of 250 and 500 mg/kg cipro. In the subchronic experiment, the animals were separated into two groups: the 1st group was control and 2nd groups were administered 125 mg/kg cipro for two weeks. The acute treatment at a dose of 500 mg/kg resulted in a significant increase in AST (alanine aminotransferase), ALT (aspartate aminotransferase), Mg (magnesium), and Ca (calcium), as did the subchronic trial at a level of 125 mg/kg. Acetylcholinesterase inhibition was measured at ciprofloxacin dosages of 250 and 500 mg/kg in the acute trial, as well as 125 mg/kg in the subchronic study. Histological examination revealed mild to severe lesions in the liver and kidneys treated with 250-500 mg/kg. The dose of 125 mg/kg resulted in significant coagulative necrosis of liver cells, sinusoidal enlargement, and severe inflammatory cell infiltration. Severe coagulative necrosis of the epithelial cells lining the renal tubules, and glomerular atrophy were all observed. Immunohistochemistry for GFAP in brain tissue showed a high positive result. We concluded that high doses of ciprofloxacin caused obvious biochemical and histological abnormalities in the liver and kidneys, cholinesterase inhibition in response to kidney and liver injuries, and increased glial fibrillary acidic protein (GFAP) expression in the brain.

INTRODUCTION

Ciprofloxacin is one of the fluorinated quinolone derivatives it is a primary metabolite of enrofloxacin (Badawy *et al.,* 2021). Ciprofloxacin is characterized by its broad spectrum and is used against a large number of negative and positive bacteria, It is also used in the treatment of most bacterial infections in chickens as well as in animals (Millanao *et al*., 2021; Rashid et al., 2023). The most important of these infections in the urinary system are those caused by Escherichia coli (E-coli) and infections of the respiratory system, bones, joints, and skin (Moustafa *et al.,* 2019). The pharmacokinetics of ciprofloxacin, which was studied in Laboratory animals are significantly different from what is observed in humans and animals, and the reason, is due to the difference in the physiological and biochemical nature of each group (Xu *et al.,* 2023; Jam et al., 2017).. The distribution of ciprofloxacin in the target organs is at a higher concentration than in the rest of the organs, as its concentration in

the liver, kidneys, and bones is relatively high (Tomas *et al.,* 2019). Given Chickens ciprofloxacin at a dose of 10 mg/kg for 5 consecutive days recorded levels of 1.6 mg/kg in the kidney and 0.18 mg/kg in the liver (Al-Snafi, 2016). Ciprofloxacin is excreted through the kidneys by glomerular filtration and tubular secretion (Landersdorfer et al, 2010; Kanval et al., 2024).

Differences in the functions of liver enzymes occur in 2-3% of patients who take quinolones, in addition to differences in the function of the liver itself in 0.3-0.9% of patients. High doses of it cause a significant decrease in enzyme activity. Alkaline phosphatase in the blood plasma of rats (Van Bambeke *et al..,* 2005; Jam et al., 2011). There is few information on the effect of using the drug in high doses in poultry. Ciprofloxacin has a genitourinary side effect that appeared in patients taking treatment with ciprofloxacin, and the epidemic of loss of kidney function was 3-7 days after treatment. Treatment in some patients over the age of 50 (Ten Doesschate ., 2022). Studies have shown that ciprofloxacin is related to the appearance of hematuria and the formation of crystals in the basal urine of laboratory animals (Krishnan *et al*., 2024). Rare cases of hematuria, interstitial nephritis, and acute kidney failure have also been recorded, as the kidney usually returns to its function several weeks after stopping treatment (Esteras *et al.,* 2020).

In this study, we sought to know the pathological toxicological effect of ciprofloxacin on the liver and kidneys of chickens in terms of pathological and histological changes, as well as measuring the level of the ALT and AST enzymes to determine the extent to which liver function is affected, as well as measuring the level of Ca and P, and percentage of cholinesterase inhibition and GFAP # in relation to the damage to the kidneys and liver.

MATERIALS AND METHODS

Animals

This experiment used chicks old 2-3 week that were raised in the animal house and fed with concentrated feed throughout the experiment, the animals were put in stander condition in dark and light 12\12 h.

Ethical approval

This study received ethical approval from the College of Science, Chemistry Department, University of Mosul.

Drugs

Ciprofloxacin was used as powder obtained from the General Company for Pharmaceuticals and Medical Supplies, Pioneer-Iraq. It was dissolved in distilled water.

Experimental design

Acute Experiment

The chicks in this experiment were randomly divided into three groups, each group consisting of 6 chicks.

- The first group was considered a control group was given distilled water only.
- The second group was given ciprofloxacin at a dose of 250 mg/kg.
- The third group of 500 mg/kg, all doses given as single i.p dose. After 24 hours had passed since the start of giving the treatment, the chicks were sacrifice by cutting the jugular vein for the purpose of collecting blood and separating the serum.

Sub chronic Experiment

The study involved splitting the chicks into two groups with each group comprising six chicks.

The first group considered a control given only distilled water

- The second group was given ciprofloxacin on a daily doses of 125 mg/kg i.p.

The treatment lasted for two weeks. Following the completion of administering the medication the young birds were euthanized by severing the vein to gather blood and organs (kidney, liver and brain) for examination. The organs were then preserved in a buffered formalin solution to prepare sections using the method to investigate any histopathological alterations.

Histopathological Method

The histopathological technique includes extracting liver and kidney tissue samples from both the treated and control groups, followed by preserving them with formalin. These samples are then sliced thinly using a microtome and dyed with stains, like hematoxylin and eosin. Afterward the slices are scrutinized under a microscope to evaluate any alterations.

GFAP Measurement in the Brain Using Immunohistochemistry

This procedure involves collecting brain tissue samples from the groups that received treatment and those, in the control group. The samples are preserved using formalin. Then sliced into sections, with a microtome. These sections undergo preparation through deparaffinization and antigen retrieval. Primary antibodies targeting GFAP are applied to the sections followed by the introduction of antibodies attached to an enzyme or fluorescent dye. After a period of incubation, the sections are. The presence and distribution of GFAP are assessed using either a microscope or a fluorescence microscope.

Kits for measuring parameter

- AST (Alanine aminotransferase) and ALT (Aspartate aminotransferase) Kits from Elabscience American Company.
- Calcium and phosphorus, Biolabo France company kits.
- Cholinesterase was measured using a modified electrometric method was used to measure modified cholinesterase (12).

Statistical analysis:

The findings were examined utilizing the SPSS software, ANOVA test, for Analysis of Variance and subsequently the results underwent, LSD testing. The outcomes were assessed with a t-test, $p \leq 0.05$.

RESULTS

The data presented in Table 1, recorded statistical analysis of the biochemical parameters individually. ALT Levels, a significant increase is observed in both treatment groups compared to the control. AST Levels also show a significant increase with ciprofloxacin treatment, particularly at the higher dose.

Mg (Magnesium) Levels significantly increase in both ciprofloxacin-treated groups compared to control, with the highest increase in the 500 mg/kg group. Ca (Calcium) Levels show a significant rise in both treatment groups.

Table 1: biochemical parameters concentrations in blood of chicks treated with ciprofloxacin.

Groups	ALT u/l	AST u/l	Mg/l ca	Mg/dl p
Control	12±0.5	18±0.2	6.4±0.1	4.7±0.5
Ciprofloxacin 250 mg\kg	16±0.5	25±0.3	8.7±0.3	9.97±0.1*

Ciprofloxacin 500 mg\kg	35±0.3*	30±0.7*	10.19±0.2*	10.18±0.2*

Values represent the mean ± standard error for six chicks/group

***The values are significantly different from the control group at the probability level p ≤0.0 5**

To analyze data presented in Table 2, The ALT levels show a significant increase in the ciprofloxacin-treated group compared to the control. AST levels also increase significantly with ciprofloxacin treatment, indicating possible hepatocellular damage. The rise, though less pronounced than ALT, still indicates a stress response or liver involvement .Magnesium levels are significantly higher in the ciprofloxacin-treated group. Calcium levels show a significant increase in the ciprofloxacin-treated group.

Table 2: biochemical concentrations in the blood serum of chickens treated with a dose of 125 mg/kg for two weeks.

Groups	ALT u/l	AST u/l	Mg/l ca	Mg/dl p
Control	11.1±0.5	15±1.2	7.5±2.5	5.5±0.6
Ciprofloxacin **125** mg\kg	19.8±0.2*	18±0.3*	12.7±3.1*	9.5±5.1*

Values represent the mean ± standard error for six chicks/group

***The values are significantly different from the control group at the probability level p ≥0.0 5**

To analyze Table 3, The changes in serum pH (ΔpH) show a slight decrease with ciprofloxacin treatment at both 250 mg/kg and 500 mg/kg doses compared to the control. However, the differences are not statistically significant ($p > 0.05$) .The changes in brain pH (ΔpH) show a significant decrease in both ciprofloxacin-treated groups compared to the control. The decrease is similar for both doses, indicating a significant effect of ciprofloxacin on brain pH.

Table 3: Values of inhibition of acetylcholine esterase in a dose of 250 and 500 mg/kg of ciprofloxacin.

Groups	ΔPH in serum\ Inhibition of acetyl choline esterase	ΔPH in brain\ Inhibition of acetyl choline esterase
Control	1.6±0.02	0.37±0.06
Ciprofloxacin 250 mg\kg	1.55±0.1	0.19 ±0.08*
Ciprofloxacin 500 mg\kg	1.5±0.08	0.20 ± 0.1 *

Values represent the mean ± standard error for six chicks/group

***The values are significantly different from the control group at the probability level p ≥0.0 5**

Table 4 show the ΔpH in serum shows a significant decrease in the group treated with 125 mg/kg of ciprofloxacin compared to the control group. This change is statistically significant ($p \leq 0.05$), indicating that ciprofloxacin has a notable effect on serum pH.

Table 4: The amount of change ΔPH in serum treated with a dose of 125 mg/kg for two weeks in successive doses.

Groups	ΔPH in serum\Inhibition of acetyl choline esterase
Control	1.8 ± 0.06
Ciprofloxacin **125** mg\kg	.05±0.04*

Histopathological Study Observations

Acute Experiment:

- **Control Group (A&D)**: **Liver (A)**: Intact hepatocytes and central vein. **Kidney (D)**: Intact glomeruli and renal tubules.
- **Ciprofloxacin 250 mg/kg Group (B&E)**: **Liver (B)**: Vacuolar degeneration of hepatocytes and congestion of the central vein. **Kidney (E)**: Vacuolar degeneration of the epithelial cells lining the renal tubules.
- **Ciprofloxacin 500 mg/kg Group (C&F)**:**Liver (C)**: Vacuolar degeneration and coagulative necrosis of hepatocytes, along with proliferation of inflammatory cells. **Kidney (F)**: Severe vacuolar degeneration and coagulative necrosis of the epithelial cells lining the renal tubules. **Staining**: H&E stain, 400X magnification (Upper panel: liver; lower panel: kidney).

Sub-Chronic Experiment:

- **Control Group (A&C)**: **Liver (A)**: Intact hepatocytes and central vein. **Kidney (C)**: Intact glomeruli and renal tubules.
- **Ciprofloxacin 125 mg/kg Group (B&D)**: **Liver (B)**: Severe coagulative necrosis of hepatocytes, expansion of sinusoids, and severe infiltration of inflammatory cells. **Kidney (D)**: Severe coagulative necrosis of the epithelial cells lining the renal tubules, detachment of renal tubules from the basal membrane, and atrophy of glomeruli. **Staining**: H&E stain, 400X magnification (Upper panel: liver; lower panel: kidney).

Immunohistochemistry of GFAP in Brain:

- **Control Group (A)**: Mild positive reaction.
- **Ciprofloxacin 125 mg/kg Group (B)**: Strong positive reaction.

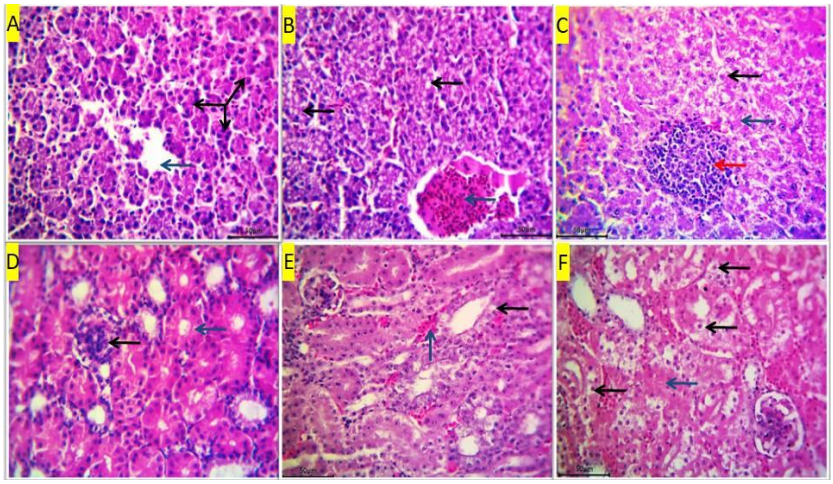

Figure 1: Histological sections of the chick's liver and kidney in the acute experiment. (A&D): Control group; (A, Liver): intact hepatocytes (black arrow) and central vein (blue arrow); (D, kidney): intact glomeruli (black arrow), and renal tubules (blue arrow). (B&E): Ciprofloxacin 250 mg/kg group; (B, Liver): vacuolar degeneration of hepatocytes (black arrow) and congestion of central vein (blue arrow); (E, kidney): vacuolar degeneration of the epithelial cells lining renal tubules (blue arrow); (C&F): Ciprofloxacin 500 mg/kg group; (C, Liver): vacuolar degeneration (black arrow) and coagulative necrosis of hepatocytes (blue arrow) and proliferation of inflammatory cells (red arrow); (F, kidney): severe vacuolar degeneration (black arrow) and coagulative necrosis of the epithelial cells lining renal tubules (blue arrow). H&E stain, 400X. (Upper panel: liver; lower panel: kidney).

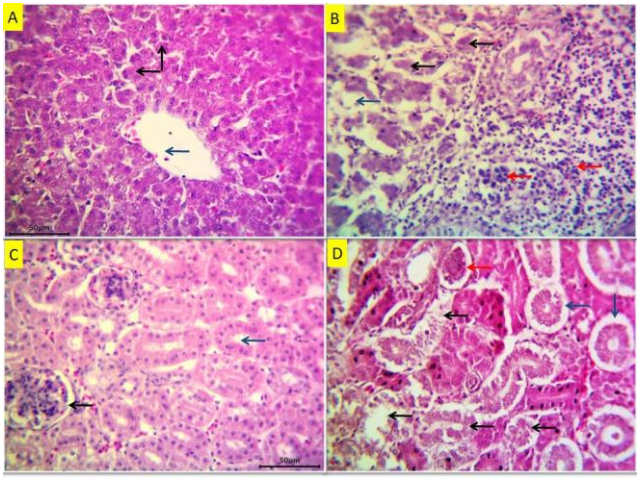

Figure 2: Histological sections of the chick's liver and kidney in the Sub-Chronic experiment. (A&C): Control group; (A, Liver): intact hepatocytes (black arrow) and central vein (blue arrow); (B, kidney): intact glomeruli (black arrow), and renal tubules (blue arrow). (B&D): Ciprofloxacin 125 mg/kg group; (B, Liver): severe coagulative necrosis of hepatocytes (black arrow), expansion of sunusoids (blue arrow) and sever infiltration of inflammatory cells (red arrow); (D, kidney): severe coagulative necrosis of the epithelial cells lining renal tubules (black arrow), detachment of renal tubules to basal membrane (blue arrow) and atrophy of glomeruli (red arrow). H&E stain, 400X. (Upper panel: liver; lower panel: kidney).

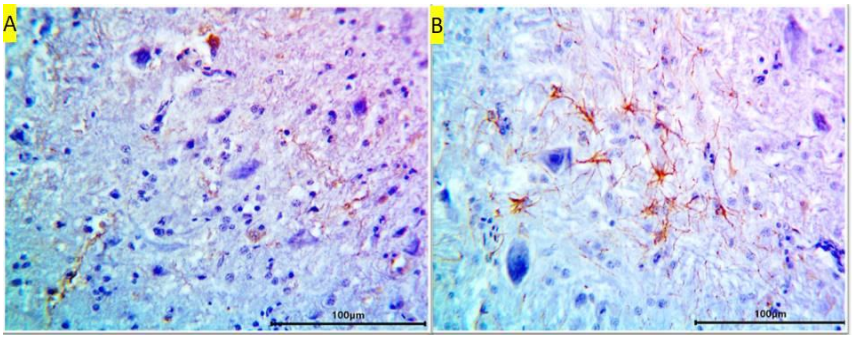

Figure 3: Immunohistochemistry expression of the GFAP of the chick's brain from (A): control group reveals mild positive reaction. (B): Ciprofloxacin 125 mg/kg group reveals strong positive reaction. (Scale-bar=100µm), 400X.

DISCUSSION

Medications, given to humans or animals in high doses, cause the body to deal with them differently, with the liver and kidneys being most effective in excreting and reducing toxic effects, leading to side effects and illnesses (Mostafa, 2017). This study discovered that administering a single dosage of ciprofloxacin shows significant increase in ALT and AST levels in ciprofloxacin-treated chicks suggests hepatotoxicity induced by the antibiotic. ALT and AST are liver enzymes, and their elevated levels are indicative of liver damage or stress (Garcia-Cortes *et al*., 2020). The dose-dependent rise, particularly marked at 500 mg/kg, indicates a correlation between ciprofloxacin dosage and liver enzyme levels. This aligns with the known side effects of ciprofloxacin, which include potential liver toxicity.The increase is more pronounced in the 500 mg/kg group, indicating a dose-dependent effect. As the growth The activity of liver enzymes shows the breakdown of liver cells in hens, but it does not provide information regarding hepatocyte function (Wang *et al*., 2020). This finding differed from (Van Bambeke et al., 2005)), which showed a decrease in ALT when mice were administered a dosage of 250 mg/kg body weight in the paw. The difference is attributable to the type of animal and the biochemical makeup of each type, or the absence of damage to the mice's

livers, as well as a possible difference in dose size. The significant increase in ALT and AST levels in ciprofloxacin-treated for 14 day, suggests hepatotoxic effects of the antibiotic. ALT is more sensitive to liver injury, which is reflected in the more substantial increase in its levels compared to AST. The significant elevation of these enzymes indicates liver stress or damage, which is a known side effect of ciprofloxacin (Zhang *et al*., 2021).

Magnesium plays a role, in biochemical reactions. The rise in magnesium levels seen during ciprofloxacin treatment could be linked to kidney issues or changes in how the body absorbs and gets rid of magnesium. The increase in magnesium levels based on dosage indicates how ciprofloxacin impacts the balance of magnesium in the body, which could affect cell functions and enzyme activities (Badawy *et al*., 2021). Calcium is essential for bone strength, muscle performance and cellular signaling. The notable increase in calcium levels among groups treated with ciprofloxacin suggests a disturbance in calcium metabolism possibly caused by increased uptake or reduced excretion. Elevated calcium levels may pose risks like hypercalcemia, influencing heart and muscle functions (Fujita and Palmieri, 2000). The results demonstrate that giving chickens a 125 mg/kg dose of ciprofloxacin for two weeks leads to shifts in biochemical markers. Higher ALT and AST levels hint at liver issues while changes in magnesium and calcium levels point to disruptions in mineral balance. These findings underscore the importance of monitoring these parameters when using ciprofloxacin to safeguard the well being of the subjects. The slight drop in serum pH during ciprofloxacin treatment indicates an impact on acid base balance, in the blood. There does not seem to be a difference, in the analysis ($p > 0.05$) between the control and treated groups indicating that ciprofloxacin at the doses examined doesn't have a significant impact on serum pH levels (Zobeiri *et al*., 2013). The drop in brain pH changes following ciprofloxacin treatment ($p < 0.05$) hints that this antibiotic can influence the acid base balance, in the brain. Both doses (250 mg/kg and 500 mg/kg) result in similar decreases, indicating that even the lower dose significantly affects brain pH. This could be due to ciprofloxacin's ability to penetrate the blood-brain barrier and its potential neurotoxic effects, which may lead to altered brain metabolism and pH regulation (Chesler, 2003).

The main purpose of measuring the change in pH is to measure the activity of the cholinesterase enzyme according to the modified Allman method, Cholinesterase is a neurotransmitter that is essential for transferring nerve messages throughout the body (Badawy et al., 2021). The result was Inhibition of the activity of acetylcholinesterase when given at a subchronic dose for two weeks. It has been demonstrated that the function of the liver and kidneys has an effective role in the level of cholinesterase in the body, as it is produced in the liver and any change in liver function results in a change in its activity, and the kidneys also play an effective role in removing it from the blood and filtering it into urine (Elizalde *et al*., 2022). The histopathological study in the acute experiment showed clear changes in liver and kidney tissues as a result of ciprofloxacin treatment. In the control group, liver cells and kidney tubules were intact without any noticeable changes. When young birds were given a dose of 250 mg/kg of ciprofloxacin they showed signs of liver cell damage, like degeneration and vein congestion. Additionally vacuolar degeneration was seen in the cells lining the kidney tubes suggesting effects on liver and kidney tissues . In another group receiving 500 mg/kg of ciprofloxacin the liver cells displayed changes including vacuolar degeneration, coagulative necrosis and an increase in inflammatory cells (Winiarska et al., 2021). The kidney cells also exhibited damage with degeneration and coagulative necrosis in the tubules lining cells. These findings indicate that higher doses of ciprofloxacin lead to toxicity levels emphasizing the importance of using caution when administering this drug at high concentrations.

Furthermore in a chronic study even a lower dose of ciprofloxacin (125 mg/kg) resulted in significant pathological changes. While liver and kidney cells remained healthy in the control group, those treated with ciprofloxacin showed necrosis, in liver cells expansion of hepatic sinusoids and substantial infiltration of inflammatory cells. In the kidneys significant tissue damage was observed, including necrosis, in the epithelial cells lining the renal tubules, detachment of renal tubules from

the basement membrane and glomerular atrophy. These findings suggest that when ciprofloxacin is administered in doses for extended periods it can lead to notable harm (Rajagopalan *et al.,* 2023). Analysis of immunohistochemistry results in the brain revealed a positive reaction in the group treated with ciprofloxacin (125 mg/kg) compared to a milder positive reaction in the control group. This implies that ciprofloxacin impacts the expression of proteins in astrocytes within the brain potentially affecting function and central nervous system integrity. These discoveries highlight effects of ciprofloxacin on liver, kidney and brain tissues in chicks (Liu *et al.,* 2021)) particularly with high doses or prolonged use. Further research is needed to establish dosage levels and understand the mechanisms behind these effects for ensuring safe and efficient utilization of this medication in avian species .

CONCLUSION

In conclusion our study indicates that administering toxic amounts of ciprofloxacin leads to biochemical and histological abnormalities, in both liver and kidney tissues. Identified by measuring the levels of the ALT and AST enzymes to determine the extent to which liver function is affected, as well as measuring the levels of Ca and P, and the percentage of cholinesterase inhibition in relation to the kidney and liver injury and, GFAP expression.

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