
Histological Effects of Ciprofloxacin on the Cytoarchitecture of the Testes of Adult male Wistar Rats

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ABSTRACT

Ciprofloxacin is an antibiotic used for the treatment of a number of bacterial infections. Several studies have shown that ciprofloxacin may impair testicular functions of male wistar rat which led to the study of its effects on the testes. Twenty (20) male wistar rats (140.52 ± 11.25) were divided into four groups ($n = 5$); control (food and water ad libitum) and experimental groups (group B received 6.25mg/kg), (group C received 12.5mg/kg), and (group D received 25.0mg/kg) of ciprofloxacin, for forty (40) days respectively through orogastric tube. 24hours after the last administration for each group, the rats were anesthetized using ignasia method. The histological result (H&E) revealed incomplete maturation of spermatozoa, widening of the interstitial spaces, loss of interstitial leydig cells and testicular atrophy of the tested groups compared to their control group.

Keywords: Ciprofloxacin, antibiotics, testes, wistar rats

INTRODUCTION

Ciprofloxacin is a synthetic anti bacteria agent belonging to the family of fluoroquinolones with very broad spectrum against microbial pathogens, especially gram negative infectious diseases, that has been approved in more than 100 countries world-wide (Wolfson and Hooper, 1985).

Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections

Laurence *et al*, (2005), and abdominal infections Solomkin *et al* (2010), among others.

Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid. (Laurence *et al*, 2005).

It also features prominently in treatment guidelines for acute pyelonephritis, complicated or hospital-acquired urinary tract infection, acute or chronic prostatitis, Brown *et al*, (2013) certain types of endocarditis, Vadakaset *et al*,(2008) certain skin infections, Falagas *et al*(2006), and prosthetic joint infections. (Oliphant *et al*, 2002).

Ciprofloxacin, like other fluoroquinolones, is known to trigger seizures or lower the seizure threshold, and may cause other central nervous system side effects. Headache, dizziness, and insomnia have been reported as occurring fairly commonly in post approval review articles, along with a much lower incidence of serious CNS side effects such as tremors, psychosis, anxiety, hallucinations, paranoia, and suicide attempts, especially at higher doses. (Heidlbaugh *et al*, 2013).

Ciprofloxacin inhibits the drug-metabolizing enzyme CYP1A2 and thereby can reduce the clearance of drugs metabolized by that enzyme. CYP1A2 substrates that exhibit increased serum levels in ciprofloxacin-treated patients include tizanidine, theophylline, caffeine, methylxanthines, clozapine, olanzapine, and ropinirole. Mostafa *et al*, (2008) stated that ciprofloxacin at dose level of 4.5 and 9.0 mg/100g body weight administered to rats for 7 and 65 days significantly decrease sperm cell motility and concentration but significantly increased sperm cell abnormalities. There was also decrease in testicular weight, seminal vesicle and prostate glands of treated rats.

This study arose due to the constant use of ciprofloxacin in the treatment of infection. The vulnerability of humans to infection cannot be over emphasized; this motivated the need to study the effects of ciprofloxacin on the reproductive system with emphasis on the testes.

MATERIAL AND METHOD

Twenty 20 male Wistar rats weighing between 150-200g were used in this study. The animals were handled according to the guidelines for animal care of the United States National Institute of Health. Normal rat chow and water was provided *ad libitum* throughout the experimental period. Ciprofloxacin was obtained from the pharmaceutical unit Abia State University Teaching Hospital Aba, Abia State. Each tablet of ciprofloxacin contains 500mg and was dissolve in 100ml distilled water. Group A was the control group and the animals did not receive any treatment while groups B, C, D were the experimental groups (group B received 6.25mg/kg), (group C received 12.5mg/kg), and (group D received 25.0mg/kg) of ciprofloxacin, for forty (40) days respectively through orogastric tube. 24hours after the last administration for each group, the rats were anesthetized using ignasia method. The testes was dissected, processed for paraffin wax embedding and routinely stained for histological study using haematoxylin and eosin technique.

RESULTS

Histological Results

The testes were examined for histological studies in the control and ciprofloxacin treated groups. In the control group, the morphology of the testes was observed.

Sections of the testes of control group showed normal histology of the testes. The interstitial spaces in-between the tubules contain Leydig cells (Ly) and some blood vessels (bv) (H&E $\times 150$). The flagella of

mature sperms are seen in the lumen of the tubules (FL), Sertoli cells (S), primary spermatocyte (Sp), rounded spermatids (Sd) (H&E x600).

Sections of the testes of group B treated with 6.25mg/kg of ciprofloxacin showed incomplete maturation of spermatozoa.

Sections of the testes of group C treated with 12.5mg/kg of ciprofloxacin showed Sertoli cells resting on the basement membrane (S). primary spermatocyte (Sp), round spermatid (Sd) resting on thin, irregular basement membrane (BM) (H&E x150). The spermatogenic cells are noticed; primary spermatocyte (Sp), round spermatid (Sd) elongated sperms (arrow) (H&E x600)

Sections of the testes of group D treated with 25.0mg/kg of ciprofloxacin showed Some seminiferous tubules with irregular outline (arrow), and acidophilic exudate in their lumen and interstitial space (*). Interstitial cells of Leydig (Ly) and congested blood vessel (bv) are noticed (H&E x150). Some seminiferous tubules with irregular outline (arrow), and acidophilic exudate in their lumen and interstitial space (*). Interstitial cells of Leydig (Ly) and congested blood vessel (bv) are noticed (H&E x600).

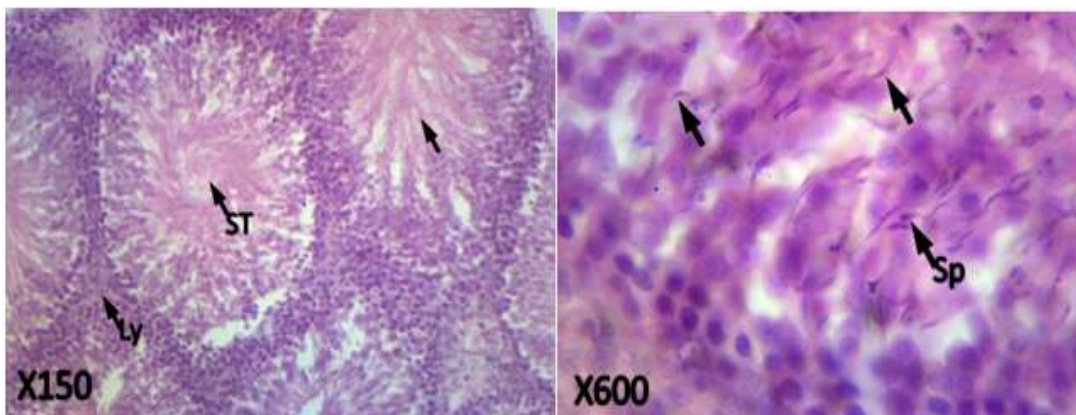


Fig: 1 Control (group A) plate 1a X150 Control (group A) plate 1 b X600

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Cross section of the testis of Group A. (Control) X 150 Magnifications. Showing normal testicular tubules with normal interstitial spaces intact
Cross section of the testis of group A (control) x 600 magnification. Showing normal testicular tubules with normal maturation of spermatozoa

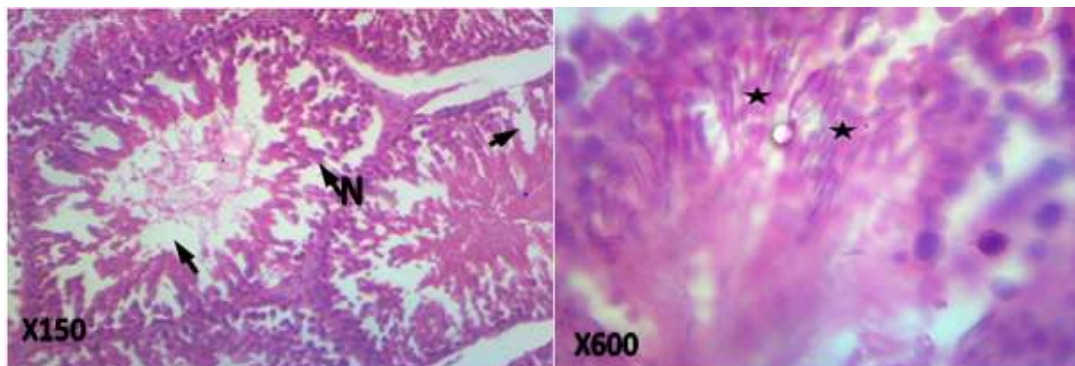


Fig. 2 Group B plate 2 a x150

Group B Plate 2 B X600

Cross section of the testis of group B (low dose of Ciprofloxacin (6.25mg/kg) X150 magnifications showing widening of some of the interstitial spaces with loss of interstitial cells it also shows disruption and necrosis (N) of the spermatogonium and there sertoli cells.

Cross section of the testis of group B (low dose Ciprofloxacin 6.25mg/kg) X600 magnifications showing incomplete maturation of the spermatozoa (star).

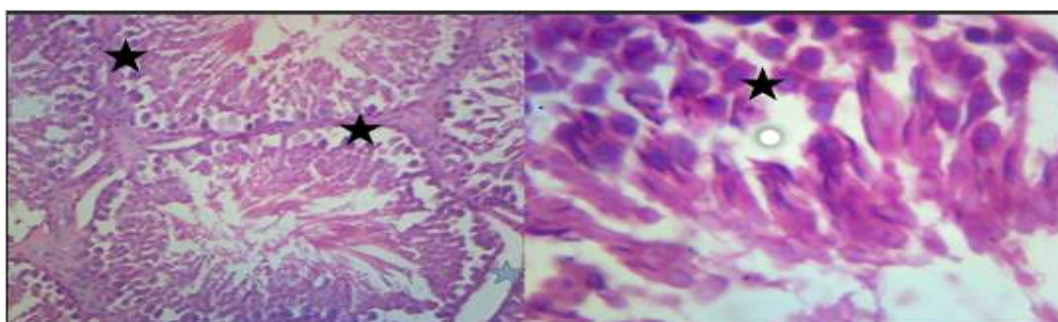


Fig: 3 Group C plate 3 a X150

Group C plate 3 b X600

Cross section of the testis of group C (minimum dose of Ciprofloxacin 12.5mg/kg) x 150 magnifications showing incomplete maturation of

spermatozoa with Sertoli cells resting on the basement membrane (S). Iry spermatocyte (Sp), round spermatid (Sd) resting on thin, irregular basement membrane (BM) (*).

Cross section of the testis of group C (minimum dose Ciprofloxacin 12.5mg/kg) x600 magnification showing incomplete maturation of spermatozoa. The spermatogenic cells are noticed; Iry spermatocyte (Sp), round spermatid (Sd) elongated sperms (*).

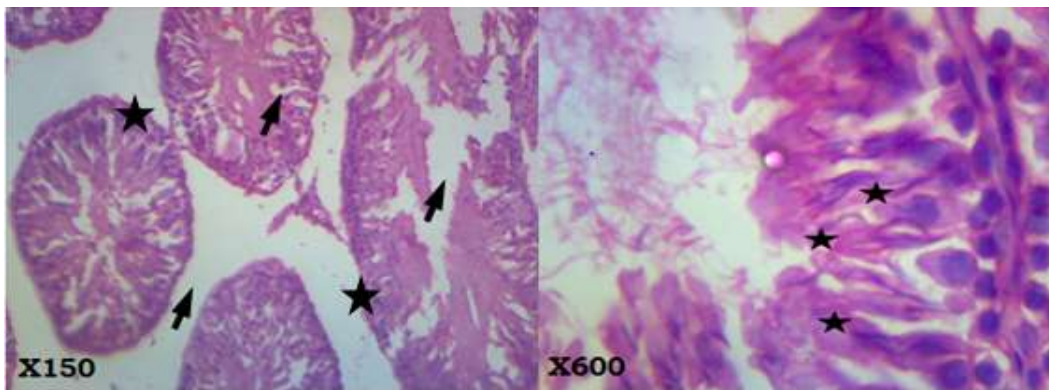


Fig: 4 Group D plate 4a X150

Group D plate 4b X600

Cross section of the testis of group D (High dose Ciprofloxacin 25.0mg/kg) x150 magnification showed that the maturation of the spermatozoa is arrested along progression and the interstitial spaces between the testicular tubules are widened as a result of testicular atrophy and some seminiferous tubules with irregular outline (arrow), and acidophilic exudate in their lumen and interstitial space (*).

Cross section of the testis of group D (High dose Ciprofloxacin 25.0mg/kg) X600 magnification. This photomicrograph shows that the maturation of the spermatozoa is arrested along progression.

DISCUSSION

In this study, the control rat testis showed multiple rounded seminiferous tubules with regular outlines. The lining epithelium consisted of Sertoli cells and other germinal cells. Sertoli cells appeared pyramidal in shape and resting on the basement membrane. The germinal epithelium consisted of spermatogonia, primary spermatocytes, rounded spermatids and elongated spermatids. The interstitial spaces in-between the tubules contained Leydig cells and some blood vessels.

In this research, L/M examination of testicular sections of treated group received 6.25 mg/kg, showed some seminiferous tubules with irregular outline. The basement appeared thin, more areas of intercellular vacuoles were detected. The interstitial space showed some Leydig cells separated by congested blood vessels. This result, it was concluded that ciprofloxacin caused a significant decrease in sperm count and motility with increased incidence of abnormal forms. Histological examination showed loosely packed stroma around seminiferous tubules with reduction in number of spermatogenic cells (Khalaf et al., 2012). However, administration of 25 mg of ciprofloxacin caused degeneration of seminiferous tubules and the spermatogenic cells. The interstitial tissue showed congested blood vessels, degenerated leydig cells. The basement membrane was thin. However, degeneration of the seminiferous tubules might be an indication for the significant reduction in the number of sperms.

The Sertoli cell tight junction is a vital component of the blood-testis barrier which is essential for normal spermatogenesis. The interrupted Sertolicells on the basement membrane of the seminiferous tubules in the groups treated with ciprofloxacin may be explained by the fact defective testicular blood barrier could cause opening of the testicular blood barrier gate. Light Microscope examination of testicular sections of the group received 25 mg/kg showed seminiferous tubules with irregular outline with acidophilic exudate in their lumen and congested blood vessels in

between. Some spermatogenic cells showed signs of degeneration; vacuolated cytoplasm and pyknotic nuclei. Increased areas of cellular loss and vaculation in the tubules. The results of this study revealed that the cross section of the testis of the treated rats showed incomplete maturation of the spermatozoa, degeneration of the seminiferous tubules and widening of the interstitial spaces as compared to the control group. The atrophy of the seminiferous tubules and incomplete maturation of sperm cells was also reported by (Khaki *et al.*, 2008) Previous study showed that caspase-3 which plays important role in apoptosis may have been activated by ciprofloxacin (Oliva *et al.*, 2002); (Zhang *et al.*, 2003).

CONCLUSION

In conclusion, the results (H&E) of this experiment revealed that the sections of the testis show incomplete maturation of the spermatozoa and widening of the interstitial spaces. The widening of the interstitial spaces signifies atrophy of the seminiferous tubules.

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