Original Article

Testicular Morphological Changes Produced by Fluoroquinolones in Adult Male Albino Rats

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Abstract

Objectives: The objective of this research work was to observe the testicular morphological changes produced by fluoroquinolones in the reproductive organs of adult male albino rats, and to see whether these changes are reversible after discontinuation of the drugs.

Materials and Method: Eighty adult male albino rats weighing 200 – 300 gms were randomly selected and divided into four groups i.e. A, B, C & D, having 20 animals in each group. A, B & C, were the experimental groups & D served as control group. All the groups were further divided into sub groups 1 & 2. Three fluoroquinolones i.e. Ciprofloxacin (135 mg / kg / day), Ofloxacin (75 mg / kg / day) & Enoxacin (12.5 mg / kg/ day) were given to the groups A, B & C respectively for 42 days. Animals of group D received distilled water only. Animals of sub groups A1, B1, C1 & D1 were sacrificed on 42nd day and testicular tissue was obtained for morphological study. Animals of sub groups A2, B2, C2 & D2 were sacrificed on 84th day and testicular tissue for morphological changes was taken. No of leydig cells, height of epithelium and diameter of seminiferous tubules were taken as experimental parameters for morphological changes.

Results: The study indicated statistically significant ($P < 0.05$) decrease in height of epithelium, diameter of seminiferous tubules and no. of leydig cells in experimental groups as compared to the control groups.

Conclusion: The changes observed in morphology could lead to decrease in sperm count and testosterone levels. This study suggests gonadotoxic potentials of fluoroquinolones and adds concern to the indiscriminate and widespread use of fluoroquinolones and recommends more rational use of these drugs.

Key words: Fluoroquinolones, Morphological changes, Gonadotoxicity.

Introduction

A decline in male fertility has been observed over the past 50 years. The quality of semen regarding the sperm count has been deteriorated. The biological significance of this change is supported by a concomitant increase in the incidence of genitourinary abnormalities such as cryptorchidism, hypospadias and testicular cancer suggesting a growing impact of the factor with serious effects on male gonadal function.[1] Environmental factors, drugs and chemicals are likely to cause changes in semen quality. The environmental factors also include the misuse of drugs and chemicals.[2] The effects of diet on reproductive potentials of males are not that much elaborated. However, the hypothesis that the nutritional specific factors can cause changes in semen quality is also supported by emerging literature.[3-6] The studies on animals suggest that the male

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Contribution`

All Authors have contributed in Study Design, Data Collection, Data Analysis, Data Interpretation, Manuscript Writing and Approval.

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fertility may be affected by dietary factors.[7-9] Antibiotic prescriptions are most commonly observed in most of the unrelated conditions which is also observed in the patients who attend the fertility clinics.[10] Patients who acquire assisted conception show indication of reproductive tract infection.[11,12] Ciprofloxacin is usually used by andrologist, urologists, and fertility specialists prior to the in vitro fertilization procedures for the bacterial infections and also to combat the increased concentration of leukocytes in the semen regardless the evidence of bacterial infection is found or not.[13]

Ciprofloxacin belongs to fluoroquinolones which is synthetic antibiotic. The fluoroquinolone antibiotics are an important, frequently prescribed group of medications. The recommended indications for fluoroquinolones include skin & soft tissue infections (SSTI), respiratory tract infections (RTI) sexually transmitted diseases (STD), and urinary tract infections (UTI). Since the introduction of fluoroquinolones in 1980's, one hundred million patients or even more have received the agents.[15] UTIs are most commonly treated with fluoroquinolones in North America and Western Europe.[16] Expanded-spectrum quinolones[17] which include norfloxacin and ciprofloxacin are highly active for eradication of bacteriuria in UTIs (> 90% cases) and gram negative bacteria.[18] Fluoroquinolones are also effective against agents of atypical pneumonia i.e. chlamydia and mycoplasma and against intracellular pathogens such as legionella and some mycobacteria, including mycobacterium tuberculosis and mycobacterium avium complex. As a class, fluoroquinolones are generally well tolerated and safe. The most commonly detected adverse effects of fluoroquinolones include the CNS disturbance, GI reactions and skin irritations. The adverse effects of newer fluoroquinolones are dose dependent having steep dose response relationship.[19]

The present study was aimed at defining the testicular morphological changes produced by fluoroquinolones such as ciprofloxacin, ofloxacin and enoxacin. No. of leydig cells, height of epithelium and diameter of seminiferous tubules were taken as experimental parameters for morphological changes and whether these changes are reversible after discontinuing the drugs.

Materials and Methods

An experimental study was conducted at Pharmacology Department of PGMI, Lahore. The total duration of study was 12 weeks. 80 male albino rats aging 7 weeks and weighing 200 to 300 grams were selected. Random division of rats was done into 4 groups named A, B, C & D with 20 animals in each group. Groups A, B & C were experimental and group D served as control. A further division of all the groups was done into sub groups 1 and 2. Three drugs, Ciprofloxacin (135 mg/kg/day), Ofloxacin (72 mg/kg/day) and Enoxacin (12.5 mg/kg/day) were given to the groups A,B and C respectively for 42 days. All the drugs were dissolved in distilled water before administration. The control group D was given distilled water only. Animals of sub groups A1, B1, C1 and D1 were sacrificed on 42nd day and testicular tissue was obtained for morphological studies. While animals of sub groups A2, B2, C2 and D2 were sacrificed on 84th days and testicular tissue was obtained for morphological studies. Morphological changes were evaluated by considering no. of leydig cells, height of epithelium and diameter of seminiferous tubules. Statistical analysis was done by using SPSS V. 16. ANOVA was applied for comparison among the various groups while P value less than 0.05 was considered significant.

Results

The mean height of epithelium was 180.73 ± 18.42 μm in A1, 169.72 ± 23.83 μm *in* A2, 151.55 ± 25.90 μm in B1, 138.49 ± 28.15 μm in B2, 161.28 ± 13.59 μm in C1, 149.76 ± 13.39 μm in C2, 272.64 ± 39.22 μm in D1 and 274.43 ± 35.28 μm in D2. In control groups the mean height of epithelium was statistically higher as compared to experimental groups sacrificed at 42nd and 84th day, p-value < 0.05. The pairs A1 vs. A2, A1 vs. C1, A2 vs. B1, A2 vs. C1, A2 vs. C2, B1 vs. B2, B1 vs. C1, B1 vs. C2, B2 vs. C1, B2 vs. C2, C1 vs. C2 and D1 vs. D2 were insignificant however other pairs were significant (Table 1).

The diameter of seminiferous tubules was 210.17 ± 9.70μm in A1, 216.67 ± 6.99 μm in A2, 206.79 ± 12.17 μm in B1, 193.68 ± 9.26 μm in B2, 190.79 ± 13.34 μm in C1, 191.84 ± 13.56 μm in C2, 256.28 ± 4.14 μm μm in D1 and 260.42 ± 9.33 μm in D2. In

control group the mean diameter of seminiferous tubule was statistically higher as compared to experimental groups sacrificed at 42nd and 84th day, p-value < 0.05. The pairs A1 vs. A2, A1 vs. B1, B2 vs. C1, B2 vs. C2, C1 vs. C2, and D1 vs. D2 were insignificant however other sub groups were significantly different (Table 2).

Table 1: *Statistical Comparison of Height of Epithelium ANOVA Table.*

	Sum of Squares	d.f	Mean Square	F	Sig.
Between Groups	209727.980	7	29961.140	43.427	.000
Within Groups	49673.667	72	689.912		
Total	259401.646	79			

Table 2: *ANOVA Table.*

	Sum of Squares	d.f	Mean Square	F	Sig.
Between Groups	1357935.555	7	193990.794	73.608	.000
Within Groups	189752.771	72	2635.455		
Total	1547688.325	79			

The number of leydig cells was 16.33 ± 2.16 in A1, 13.83 ± 1.21 in A2, 13.96 ± 2.58 in B1, 13.23 ± 2.96 in B2, 12.96±2.84 in C1, 12.96 ± 2.27 in C2, 20.23 ± 5.16 in D1 and 22.26 ± 5.08 in D2. In control group the mean number of leydig cell was statistically higher as compared to experimental groups sacrificed at 42nd and 84th day, p-value less than 0.05. The sub groups A1 vs. C1, A1 vs. C2, A1 vs. D1, A1 vs. D2,

Table 3: *ANOVA Table.*

	Sum of Squares	d.f	Mean Square	F	Sig.
Between Groups	915.839	7	130.834	11.980	.000
Within Groups	786.333	72	10.921		
Total	1702.172	79			

A2 vs. D1, A2 vs. D2, B1 vs. D1, B1 vs. D2, B2 vs. D1, B2 vs. D2, C2 vs. D1, and C2 vs. D2 were statistically significant however remaing sub groups were insignificant. ***(Table # 3)***

In majority of control group animals (D1& D2) the lumen of the seminiferous tubules were filed by mature spermatozoa. All the cells of spermatogenic series and sertoli cell were healthy looking. The interstitial tissue was normal, richly vascularised and occupied by acitive healthy looking leydig cell (Fig 1, 2).

In fluoroquinolones exposed groups (A1, A2, B1, B2 & C1, C2) the testicular tissue showed dis-organized tubules with variable diameter, the basement membrane was thickened, intact and appeared hyalinized. Various types of architectural derangements like vacuolization, karyolysis and cytolysis were observed in almost all the tubules (Fig. 3, 4, 5, 6, 7, and 8).

Most of the tubules were devoid of spermatids and the number of cell layers was reduced. The scattered early stage of spermatogenic cells were seen in the seminiferous tubules. The shed spermatogenic cells debris was seen in the lumen of a large number of seminiferous tubules (Fig. 4, 6, 8).

The sertoli cells showed degeneration along with degeneration of interstitial leydig cells, they were reduced in number and exhibited pyknoticnucei (Fig. 3, 4, 5, 6, 7, 8).

Discussion

The fluoroquinolones are anti-microbial agents with a broad spectrum of antibacterial activity. These are synthetic and effective after oral administration for a variety of infectious diseases. The fluoroquinolones have well documented therapeutic and adverse effects. However, the present study proposed that prolonged exposure to fluoroquinolones (ciprofloxacin, ofloxacin and enoxacin) even at therapeutic doses produced toxicity in reproductive activity of male albino rats. Morphological changes produced by fluoroquinolones in experimental groups are the evidence of such toxicity.

Degenerative changes in the seminiferous tubules are the evidence for genotoxicity. This is significant and remains high in experimental groups A2, B2, C2 as compared to control group D2 which indicates that

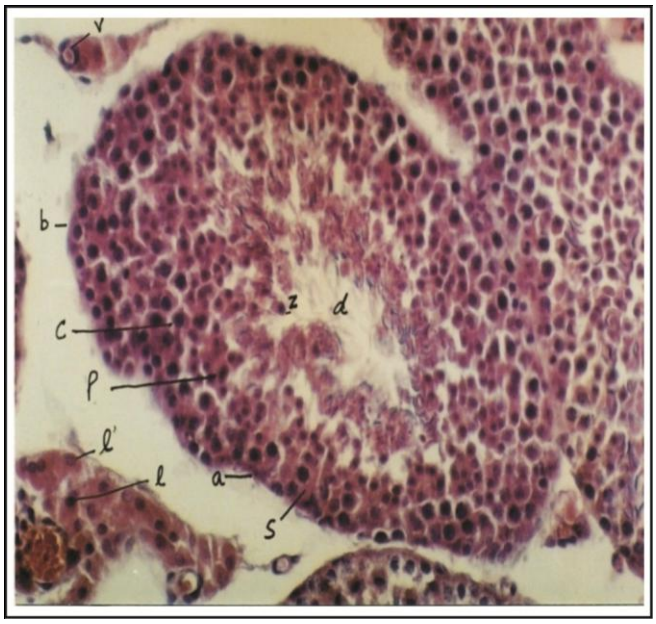

Fig. 1: *Photomicrograph Normal Rat Testes Group D_1*
Showing

a	Spermatogonia	d	Lumen of Tubule
p	Spermatocyte	b	Basement membrane
c	Spermatid	l	Leydig Cell
z	Spermatozoa	v	Blood Vessels
s	Sertoli cell		

Staining H&E Magnification x 40

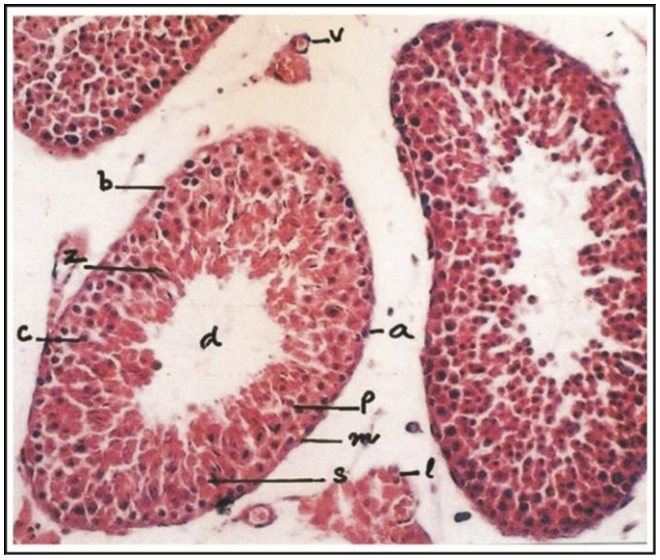

Fig. 2: *Photomicrograph Normal Rat Testes Group D_2*
Showing

a.	Spermatogonia	d.	Lumen of Tubule
p.	Spermatocyte	b.	Basement membrane
c.	Spermatid	l.	Leydig Cell
z.	Spermatozoa	v.	Blood Vessels
s.	Sertoli cell		

Staining H&E Magnification x 40

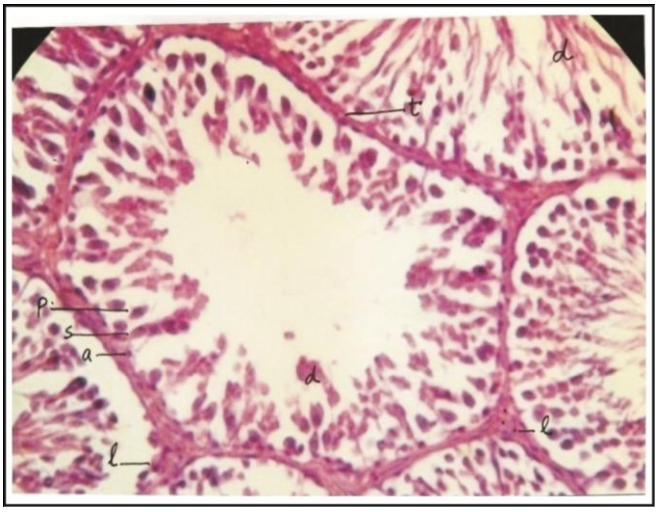

Fig. 3: *Photomicrograph of Rat Testes Group A_1 Exposed to Ciprofloxacin & Sacrificed at 42nd day.*
Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

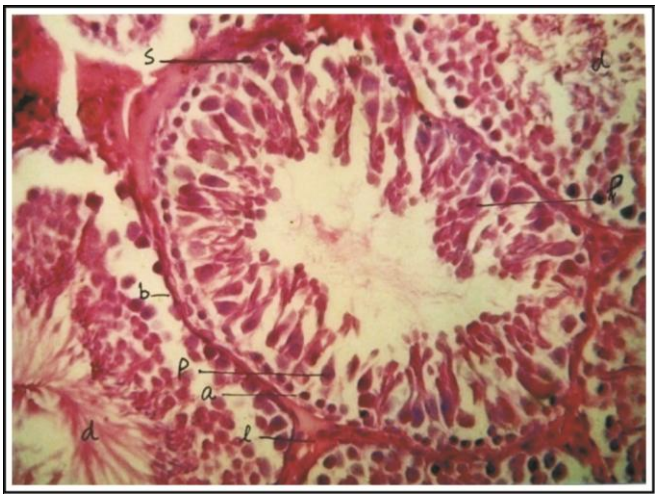

Fig. 4: *Photomicrograph of Rat Testes Group A_2 Exposed to Ciprofloxacin& Sacrificed at 84th Day.*
Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

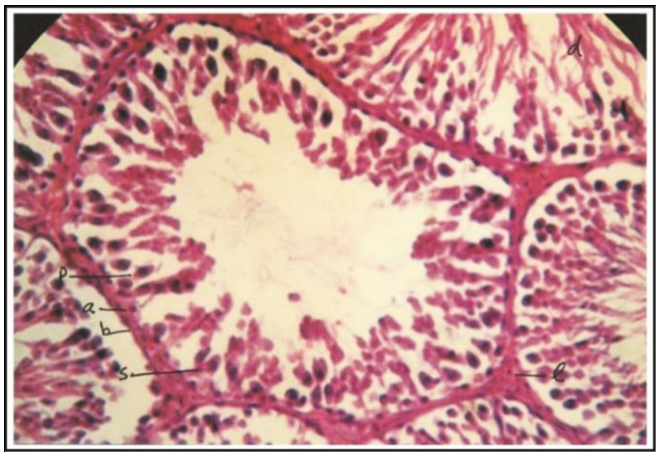

Fig. 5: *Photomicrograph of Rat Testes Group B_1 Exposed to Ofloxacin & Sacrificed at 42nd Day.*

Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

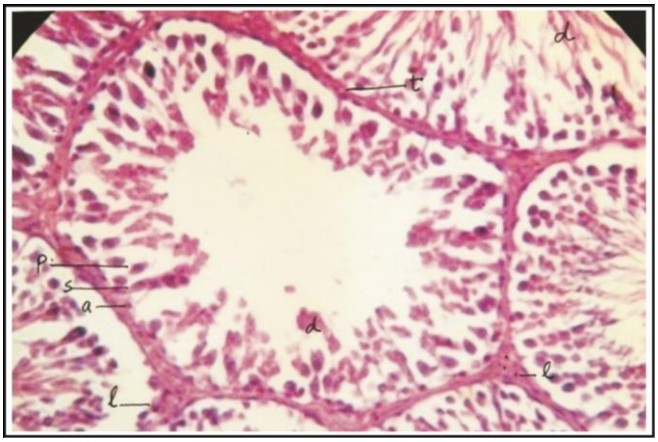

Fig. 7: *Photomicrograph of Rat Testes Group C_1 Exposed to Enoxacin & Sacrificed at 42nd Day.*

Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

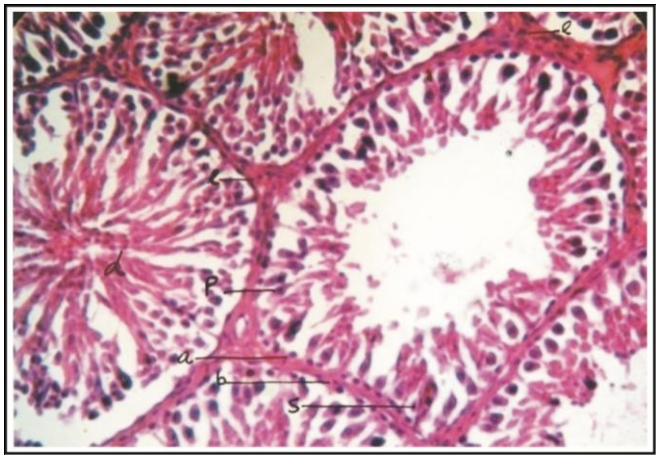

Fig. 6: *Photomicrograph of Rat Testes Group B_2 Exposed to Ofloxacin & Sacrificed at 84th Day.*

Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

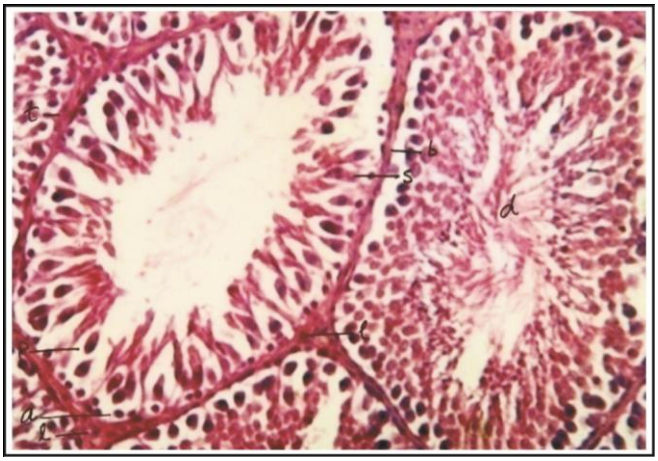

Fig. 8: *Photomicrograph of Rat Testes Group C_2 Exposed to Enoxacin & Sacrificed at 84th Days.*

Showing:

t. shrunken tubules with wavy outlines
b. thickened and hyalinized basement membrane
a. spermatogonia undergoing kariolysis and karyorrhexis
p. degenerating spermatocyte
s. degenerating sertoli cell
d. lumen with cellular debris
l. leydig cell showing degenerative changes

Staining with H&E Magnification 40 X

changes are permanent. These results indicate that fluoroquinolones like other chemical agents may directly interfere with the process of spermatogenesis. These were in agreement with other studies that showed fluoroquinolones such as Ofloxacin, Enoxacin, and ciprofloxacin have negative effects on testis architecture in rats.[20-22] In this study mean diameter of seminiferous tubules was higher in group D (Control) in contrast to A, B and C (experimental groups) $P < 0.001$ which is significant, also shown in photomicrographs Fig. 1 to 8. Studies using rats treated with therapeutic doses of fluoroquinolones confirmed the observation in humans regarding the adverse effect of fluoroquinolones on spermatogenesis.[23]

Changes observed on light microscope include expansion of interstitial and intertubular space with vacuolization, degeneration and necrosis of interstitial (leydig) cells following which an exudation into the interstitium was developed and congestion in veins increased in all experimental groups as compared to control groups showing P value < 0.05 that is significant. Multiple comparison among study groups showed that Ciprofloxacin produced less morphological changes (Fig. 3 & 4) in testicular tissue as compared to Ofloxacin (Fig. 5 & 6) and Enoxacin (Fig. 7 & 8). These data were confirmed when compared with previous study conducted by khaki et al in 2008.[20] Anderson et al (2012) showed that Ofloxacin at a dose of 72 mg/kg per day had almost the highest potential in terms of impairment of the rat testicular function.[24] Other studies also added that fluoroquinolones have toxic effects on testicular functions of different animals.[21,25-28]

Conclusion

Evidence of morphological changes produced by fluoroquinolones in this study showed that these changes could lead to decrease in sperm count and serum testosterone levels. This study suggests that fluoroquinolones are potentially gonadotoxic therefore widespread and indiscriminate use of quinolones shall be discouraged.

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