Elite Journal of Laboratory Medicine. Volume 2 Issue 8(2024), Pp. 23-32
https://epjournals.com/journals/EJLM

Experimental Evaluation of the Effects of Ciprofloxacin and Levofloxacin on Semen Parameters of Male Rats

SO Onemu [1*], JT Kolawole [2], CN Isibor [3] and FG Ademulegun [4]

1. Samson O. Onemu, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria. onemuso@achievers.edu.ng; samsononemu@gmail.com. ORCID 0009 0005 7247 9334.
2. Justus T. Kolawole, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria. jutustolutope@gmail.com.
3. Clement N Isibor Department of Biological Sciences. University of Delta, Agbor, Nigeria. Clement.isibor@unidel.edu.ng.
4. Faith G. Ademulegun, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria. Faithademulegun@gmail.com.

*Corresponding author: Samson O. Onemu, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria. onemuso@achievers.edu.ng; samsononemu@gmail.com. ORCID 0009 0005 7247 9334.

Abstract

Diverse range of adverse effects in the administration of fluoroquinolones that includes negative impacts on semen parameters abound in literature. The current study evaluated the effects of ciprofloxacin and levofloxacin on the semen indices of experimental rats. Groups of ten rats each administered with standard doses of ciprofloxacin and levofloxacin twice daily for seven days and a third group with sterile distilled water as control. Examination of semen samples from the cauda epididymis on day 35 after the agents' administration revealed significant declines in sperm cells motility ($P<0.0001$); significantly increased abnormal sperm cells morphology ($P<0.0001$) as well significant declines in sperm cells concentrations were recorded for both ciprofloxacin and levofloxacin. This study illuminates the harmful effects of these fluoroquinolones on male fertility. There is therefore, a need for careful assessment of the potential harm versus benefits in the use of these antimicrobial agents, otherwise preference for substitute agents especially in men with fertility challenges is desirable.

Keywords: *adverse effects, fluoroquinolones, male fertility, semen parameters.*

Citation: Onemu SO, Kolawole JT, Isibor CN, Ademulegun FG. Experimental Evaluation of the Effects of Ciprofloxacin and Levofloxacin on Semen Parameters of Male Rats. Elite Journal of Laboratory Medicine, 2024; 2(8): 23-32

1. Introduction

The challenge of infertility affects 30-50% in different population settings [1]. Male infertility is responsible for up to 20% of infertility separately and a further 30-40% of the partner's infertility [2]. A number of etiological factors contribute to male infertility that range from urinary tract infections, UTIs, anatomical abnormalities, occupational and environmental hazards such as radiation, smoking, scrotal temperature, heavy metals and the administration of antimicrobial therapeutic agents [3,4]. There are reports of fluoroquinolones, FQs adversely impacting male fertility [4,5]. The fluoroquinolones are some of the commonest antimicrobial therapeutic agents prescribed for a wide variety of infections globally [6,7,8,9,10]. The wider activity range of FQs against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa* and *Mycobacteria* species with the added advantage of oral formulations that possess excellent bioavailability contributes to the passion for their regular use [11,12]. Fluoroquinolones have been administered widely for infections in the last four decades since introduction into the clinical arena and may remain so for the foreseeable future [13,14]. The activity of the FQs is directed at the bacterial DNA gyrase and topoisomerases II and IV (Topo II and Topo IV) which are essential enzymes in DNA replication in both prokaryotes and eukaryotes [7,15]. Individually, TOP I and TOP II work in opposition to maintain the super-coiled structure of the DNA, while Topo I reduces the negative twist to allow unwinding into a relaxed state and alter the topology thus permitting replication [7,16-18]. Topoisomerase IV enables the newly synthesized DNA strands to disassemble synchronously [19,20]. The impressive outcome of the fluoroquinolones in treating bacterial infections accompanied with wide range of unknown adverse drug effects surfaced during the post-marketing data collection period [21]. Some of the ADEs are a major source of unease as they include neurological complications [22]. The fluoroquinolones exhibit extensive multiplicity of ADEs that range from tendinopathy, tendon rupture, arthropathy, and interference with glucose metabolism, depression and central nervous system, CNS disturbances [23-26]. Others ADEs include teratogenic effects on embryo, abnormal semen parameters and chromatin fragmentation [3-5,27-29]. The planning of the current study was to evaluate the effects of ciprofloxacin and levofloxacin on the fertility of experimental male rats.

2. Materials and Methods

2.1. Ethical Consideration

The Ministry of Agriculture and Forestry, Akure, Ondo State gave ethical clearance for the study with letter Ref. No. MNR/V.384/80 issued June 14, 2024, for strict compliance with the guidelines in the care and use of animals for research.

2.2 Experimental Animals

Adult Wistar rats (30) grouped randomly into A, B and C of 10 rats each for the administration of ciprofloxacin and levofloxacin and 10 as control.

2.3 Study Design.

Experimental study to evaluate the effects of two regularly prescribed fluoroquinolones - ciprofloxacin and levofloxacin on the potential harmful effects of the agents on the reproductive capacity of male rats as a measure of toxicity.

2.4 Experimental Procedure.

The rats were allowed to rest for 5 days to acclimatize, weighed and to determine the concentration of agent to be administered. The rats assigned into three groups in cages boldly marked (Group A-C). Group C served as the control group and received only normal rat feed and water. Group B received 500 µg/kg of ciprofloxacin administered orally twice daily for 7 days. Group C received 200 µg/kg of levofloxacin orally twice daily for 7 days with the aid of a gavage.

2.5 Examination of rats.

Each male rat was initially anesthetized with diethyl-ether before being euthanized through cervical dislocation and orchiectomy performed via the castration method, involving a midline or pre-scrotal incision to access the testicular region. The testes extruded through the incision site, and the tunica vaginalis incised to expose the testicular tissue. Each spermatic cord identified, ligated, sutured and transected. The method of semen collection is similar to that described by Okoye et al, [30]. Seminal fluid was from the caudal epididymis was teased into a micro-tube for prompt analysis to maintain to optimum semen quality.

2.6 Laboratory Analysis

Wet mount of seminal fluid accomplished by the addition of an equal amount of phosphate buffered saline, PBS solution (1:1 dilution) to enhance sperm cells motility and evaluation for morphology. The mixture was then gently vortex-mixed to preclude the introduction of bubbles and foam, from which a small aliquot (5 µL) placed carefully on a clean glass slide, covered with a coverslip to prevent evaporation and preserve specimen integrity. The prepared slide was then examined under a bright-field microscope (x10 and x40), enabling the accurate assessment of motility, morphology and sperm concentration applying standardized guidelines [31].

2.7 Statistical Analysis

Statistical Package for Social Science Version 20 (SPSS V.20) software deployed for the analysis of data with use of descriptive statistics, including mean, standard deviation, inferential statistics and *t*-test to determine significant differences test rats and control rats. The confidence limit was set at 95%, ($P<0.05$).

3. Results

Table 3.1 shows the mean percent of sperm cells motility and morphology parameters of each experimental group of rats with the control set of rats showing the most impressive indices - 78.2 ± 3.5% and 85.1 ± 2.1% for motility and sperm cells with normal morphology individually. Rats treated with ciprofloxacin and levofloxacin showed sperm cells motility and normal sperm cells forms of 52.1 ± 5.2% and 61.3 ± 4.5%, and 53.5 ± 5.5% and 62.5 ± 4.2% respectively. Each of the agents showed a significant level of decline ($P<0.0001$) in both motility and in the proportion of normal sperm cells. Table 3.2 represents a comparative analysis of the mean sperm cell concentration of the three groups of rats. The spermatozoa concentration in the control group of rats was significantly higher ($P<0.0001$), 52.1 ± 1.75 x 10^6/mL compared to 33.1 ± 3.43 x 10^6/mL and 33.2 ± 3.25 x 10^6/mL for ciprofloxacin and levofloxacin treatment groups respectively.

Table 3.1: Assessment of sperm cells motility and morphology after administration of agents.

Group of rat	Motility (%)	Normal sperm cells (%)	P-value
Ciprofloxacin	52.1 ± 5.2	61.3 ± 4.5	<0.0001
Levofloxacin	53.5 ± 5.5	62.5 ± 4.2	<0.0001
Control	78.2 ± 3.5	85.1 ± 2.1	

Table 3.2: Concentration of sperm cells of rats treated with ciprofloxacin and levofloxacin

Group of rat	Sperm cells concentration (x 10^6/mL)	P-value
Ciprofloxacin	33.1 ± 3.43	<0.0001
Levofloxacin	33.2 ± 3.25	<0.0001
Control	52.1 ± 1.50	

4. Discussion

The study uncovered the detrimental effects of ciprofloxacin and levofloxacin on the fertility of the experimental male rats. Sperm cells motility declined significantly (P<0.0001) in male rats administered with ciprofloxacin (Table 3.1). Rats that received levofloxacin similarly demonstrated significant reduction (P<0.0001) in the proportion of motile sperm cells compared to the control group of rats. The abnormalities in sperm cells morphology similarly increased significantly (P<0.0001). The harmful effects of ciprofloxacin and levofloxacin point to the deleterious impacts of ciprofloxacin and levofloxacin on male reproductive capacity reported from earlier studies [32-35]. The administration of each of ciprofloxacin and levofloxacin in the experimental rats caused significant reduction (P<0.0001) in sperm cells concentration. This is an indication that the use of these fluoroquinolones leads to the induction of deleterious events in the male reproductive system. This is consistent with observations from previous studies [3,24-28]. The pronounced impact of ciprofloxacin and levofloxacin on semen parameters from this study cannot be divorced from the mode of action of the fluoroquinolones which is directed at the inhibition of bacterial DNA gyrase and topoisomerases II and IV (TOPO II and TOPO IV) necessary for replication of bacteria. Topoisomerase I (TOPO I) and TOPO II are critical enzymes in both prokaryotes and eukaryotic organisms that function synchronously during replication. The inhibition of TOPO II indirectly hampers the activity of TOPO I that may reflect the harmful effects seen in mammalian cells [17,18,20]. The increased levels of abnormalities recorded from this study lays credence to reports of DNA fragmentation and other abnormalities reported from prior studies on the adverse effects of fluoroquinolones [5,16,19]. This represents the most direct evidence that ciprofloxacin and levofloxacin are not sufficiently selectively toxic to only prokaryotic cells or bacteria but also to mammalian cells, and therefore, capable of exacerbating male infertility and complicate oligoasthenozoospermia. It is instructive therefore, to weigh the risks-benefits ratio in the prescription of fluoroquinolones antimicrobial agents particularly ciprofloxacin and levofloxacin for males in the active reproductive age. The consideration for placement of these fluoroquinolones into the list of reserved drugs for which no alternative agent exists is desirable [4,5,19,20,36-39].

5. Conclusion

Ciprofloxacin and levofloxacin are fluoroquinolones prescribed extensively with demonstrable deleterious impacts on semen indices requiring risk-benefit assessment for their administration in males in the active reproductive years. The removal of fluoroquinolones from routine use into

agents of last resort will minimize the occurrence of adverse events and diminish the challenge of bacterial resistance to antimicrobial agents.

Funding: The authors received no funding and have no conflict of interest to declare.

Data availability: All relevant data from the current study are included and assessable on reasonable request from the corresponding author.

References

1. Eisenberg ML, Esteves SC, Lamb DJ, Hotaling JM, Giwercman A,Hwang K, et al. Male fertility, 2023; 9(49): doi.org/10.1033/s41572-023-00459-w.

2. Leslie SW, Soon-Sutton TL, Khan MA. Male fertility [Updated 2024 Feb 25]. In: StatPearls [Internet]. Treasure Island (FL), StatPearls Publishing, 2024, www.ncbi.nlm.nih.gov/book.

3. Onemu SO, Onemu-Metitiri MO, Odeyemi O, Uyigue PO, Obeagu EI. Adverse effects of antimicrobial therapeutic agents in common use: a review. Elite J Med, 2024; 2(7): 1-20.

4. Kheirandish R, Emadi L, Akhtardanssh B, Azizi S, Imani M, Mahmoodabadi F, et al. Effects of ciprofloxacin on testicular tissue and sperm quality in rabbits. Asian J Reprod, 2020; 9(2):83-88.

5. Onemu SO, Ibeh IN. The effect of quinolone chemotherapy on spermatozoal concentration of Nigerian males. Nig J Biomed Engineering, 2001; 1(1): 12-15.

6. Fabrega A, Madurga S, Giralt E, Vila J. Mechanism of action and reistance to quinolones. Microb Biotechnol, 2009; 2(1): 40-61.

7. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. Fluoroquinolones resistance: mechanisms, impacts on bacteria and role in evolutionary success. Trends Microbiol, 2009; 22(8); 438-45.

8. Aldred KJ, Kerns RJ, Osheroff W. Mechanism of quinolone action and resistance. Biochemistry, 2014, 53(10): 1565-74.

9. Correia S, Poeta P, Hebraud M, Copelo JL. Mechanisms of quinolone action and resistance: where do we stand? J Med Microbiol, 2017; 66(5): 551-559.

10. Wierzbinski P, Hubska J. Henzler M, Kucharski B, Bies R, Krzystanek M. Depressive and other adverse CNS effects of fluoroquinolones. Pharmaceuticals, 2023; 16(6): 1105, doi.org/ph16081105.

11. Bush RK, Diez-Santos I, Abbott LR, Maxwell A. Quinolones: mechanism, lethality and their contributions to antibiotic resistance. Molecules, 2020; 25(23): 5662, doi.10.3390/molecules25235662.

12. Millanao NG, Mora AI, Villagra NA, Bucarey SA, Hillago AA. Biological effects of quinolones: a family of broad-spectrum agents'. Molecules, 2021; 26(23): doi.10.3390/molecules26237153.

13. Brar RK, Jyoti U, Patil RK, Patil HC. Fluoroquinolone antibiotics: an overview. Adesh Univ J Med Sci Res, 2020; 2(1): 26-30.

14. Thompson D, Xu J, Ischia J, Bolton DM. Fluoroquinolones, resistance in urinary tract infection: epidemiology, mechanisms of action and management strategies. BJUI Compass, 2023; 5(1): 5-11.

15. Pham TD, Kerns RJ, Osheroff W. Quinolone antibiotics. Medicinal Chem Commun, 2019; 10: 1717-39.

16. Hooper DC, Jacoby GA. Topoisomerase inhibitors: fluoroquinolone mechanisms of action and resistance. Cold Spring Harb Perspect Med, 2016; 6(10): a025320.

17. Kokot M, Anderluh M, Hrast M, Monovski N. The structural features of novel bacteria topoisomerase inhibitors that define their activity on topoisomerase IV. J Medicinal Chem, 2020; 65(9): 6437-40.

18. Groosman S, Fishwick CW, McPhillie MJ. Development in non-intercalating bacterial topoisomerase IV. Pharmaceuticals, 2023; 16(2); 261, doi.org/10.3390/ph16020261.

19. Helgesen E, Saetre F, Skarstad K. Topoisomerase IV tracks behind the replication fork and SeqA-Complex during DNA replication in Escherichia coli. Sci Rep, 2021; 11: 474, doi.org/10.1938/s41598-020-80043-4.

20. Hirsch J Klostermeier D. What makes DNA topoisomerase a gyrase or a Topo IV? Nucleic Acids Res, 2012; 49(11): 6027-40.

21. Tang K, Zhao H. Quinolone antibiotics: Resistance and therapy. Infect Drug Resistance, 2023; 16: 811-20.

22. Anwar AJ, Lu L, Plaisance CJ, Daniel CP, Flanagan CJ, Wenger DM, et al. Fluoroquinolone: neurological complications and side effects in clinical practice. Cereus, 2024; 16(2): e54565.

23. Mandell L, Tillotson G. Safety of fluoroquinolones: an update. Can J Infect Dis, 2002; 13(1):54-61.

24. Aspinall CL, Good CB, Jang R, McCaren M Dung D, Cunningham HE. Severe dysglycemia with the fluoroquinolones: a class effects. Clin Infect Dis, 2009; 49(3): 402-8.

25. Daneman N, Lu H, Redelmeier DA. Fluoroquinolones associated with severe adverse events: a longitudinal cohort study. BMJ Open, 2015; 5(11): e010077

26. Kaden T, Graf K, Kennert K, Li R, Mosia SM, Raasch M. Evaluation of drug-induced liver toxicity of trovafloxacin in a human microphysiological model. Sci Rep, 2023; 13: 13338, doi.org/10.1038/s41598-023-4004-z.

27. Zhang H., Xu W, Li B. Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to moxifloxacin. Reprod Toxicol, 2012; 34(4):640-649.

28. Khaki A. Assessment on the adverse effects of aminoglycosides and fluorqinolones on sperm parameters and male reproduction: a systematic review: Iran J Reprod Med, 2015; 13(3): 125-34.

29. Mokhimor HM, Kandiel MM, Amin AA, Elsawah HK, El-Mahmoudy AM. Ciprofloxacin and levofloxacin adversely affect male fertility indicated by pharmacological and andrological evidence. IMSEAR, 2020; ID: sea-200531.

30. Okoye, E. Saikali, S.W. Orchiectomy. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. PMID: 32966007. www.ncbi.nlm.nih.gov/book.

31. World Health Organization. WHO laboratory manual for the examination and processing of human semen (5th ed), World Health Organization, Geneva, 2010: pp 7-67.

32. Zobeiri E, Sadkhanlou RA, Salami S, Mardani K, Ahmadi A. The effect of ciprofloxacin on spermatozoa DNA damage, infertility and potential early embryonic development in NMRI mice. Vet Res Forum, 2012; 3(2): 131-5.

33. Ahmadi R, Ahmadifari M, Safapour E, Vahidi-Evrisofla N, Darab M, Ali ME, et al. The effects of levofloxacin on testis tissues and spermatogenesis in rat. Cell J, 2016; 18(1): 112-116.

34. Timermans A, Vazquez R, Otero F, Gosalev J, Johnson S, Fernandez JL. Antibiotics toxicity on human spermatozoa assessed using the sperm DNA fragmentation dynamic assay. Andrologia, 2021; 54(2); e14328.

35. Baggio D. Anand-Rajah MR. Fluoroquinolones antibiotics and adverse events. Aust Prescr, 2021; 44:161-164.

36. Owens RC, Ambrose PG. Antimicrobial safety: focus on fluoroquinolones. Clin Infect Dis, 2005; 41(Suppl 2): 5144-5157.

37. Liu HH, Safety profile of the fluoroquinolones. Drug Safety, 2010; 33: 353-69.

38. Hamilton F, Darley E, McGowan A. Fluoroquinolones: clearer evidence and guidance on safety are needed. BMJ, 2024: 385q 986, doi.org/10.1136/bmj.q981.

39. Lacobucci G. Fluoroquinolones antibiotics: prescribe only as last resort, say UK regulator. BMJ, 2024; 384, doi.org/101136/bmj.q183.