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Effects of Levofloxacin on Male Reproductive System Parameters and Sperm DNA Normality in Rats

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Abstract :

Although fluoroquinolones are excessively prescribed in the therapy of genital tract infections, it can be associated with fertility problems and insufficient information concerning their effect on fertility are present. The goal of this study is to estimate the effects of levofloxacin on certain sperm function parameters (even on reproductive tissues) and to investigate whether levofloxacin can affect sperm DNA integrity or chromatin quality. Forty eight male adult were enrolled in this study. The animals were randomly divided into 6 groups; four levofloxacin treated groups which were treated with a dose of either (37.5 mg/kg/day) or (75 mg/kg/day) of levofloxacin and two control groups, for each treatment dose the treatment continue for either 14 days or 28 days. Certain epididymal sperm function parameters: sperm concentration, sperm motility and morphologically normal sperm percentage were analyzed. In addition to analysis of sperm DNA integrity and chromatin quality, histopathology of testes, epididymus and serum testosterone concentration. Sperm function parameters were not significantly affected when levofloxacin was administered for 14 days. While a significant reduction in sperm concentration, percentage of morphologically normal sperm and sperm motility were observed when the drug was administered for 28 days. A significant increase in the level of DNA fragmentation with a significant reduction in chromatin quality were also observed in levofloxacin treated groups. The testes of rat treated with levofloxacin showed changes in the number of spermatozoa in some seminiferous and epididymal tubules when the drug was applied in high doses and for 28 days. Serum testosterone concentration was not significantly affected by levofloxacin administration. These results indicate that levofloxacin could adversely affect fertility potential in male.

Keywords: Fertility, Levofloxacin, Male, Sperm DNA.

INTRODUCTION:

Antibiotics are usually prescribed in the treatment of several types of diseases. Whereas some patients requiring assisted fertilization, in some cases, they display evidence of the male reproductive tract infection [1]. Therefore, the use of antibacterial agents is essential in the treatment of male genital tract infections that can adversely affect fertility.

The antibiotic fluoroquinolones are commonly prescribed by fertility specialists in the therapy of several types of bacterial infections when high concentration of leukocytes are displayed in the semen or prior to *in vitro* fertilization program, without taking consideration to the microbial evidence of infection [2].

Infertility which represent a disease of the reproductive system can be defined as failure to achieve pregnancy after 12 months or more of a regular unprotected sexual intercourse [3]. Male infertility defect represent more than 40% of infertility problems. There are a number of factors which can affect male fertility; anatomical factors like varicocele, ejaculatory disorders or ductal obstructions, represent some male infertility factors [4]. In addition, male infertility can be resulted from abnormalities in sperm parameters; it was estimated that sperm production defects represent 35-75% of male infertility problems. Other factors that associated with male infertility are sperm antibodies, infection, radiation, reactive oxygen species, heavy metals, cigarette smoking, hormonal factors, some therapeutic drugs and others. All these causes can be attributed to abnormalities in certain sperm parameters resulting in azoospermia, asthenospermia, teratozoospermia, oligozoospermia or others [5].

Levofloxacin, third generation fluoroquinolone antibacterial agent is active against most aerobic gram-positive and gram-negative organisms with moderate activity against anaerobes [6]. Treatment by levofloxacin may cause several side effects on the central nervous system, nerves, tendons, muscles, joints, and, gastrointestinal, including nausea, vomiting, and constipation [7] . *In vivo* and *in vitro* genotoxicity studies had propose that these antibiotics are safe for therapeutic use [8] . However, other studies have demonstrated that some fluoroquinolone antibiotics such as ciprofloxacin impair both testicular structure and function [9]. Therefore, this study was designed to found out the impact of levofloxacin on some aspects of male reproductive system in rat.

MATERIAL AND METHODS

Forty eight male adult Albino-rats of the Wister strain were enrolled in this study. Their weight was in range of (200-250) gm and the age of rats was ranged between 8-9 weeks old. The rats were housed under controlled temperature around 25 °C and 13± 2 hours light-dark cycles in cages. They were fed a standard commercial pellets. After adaptation, the experimental groups were equally divided into 6 groups (4 levofloxacin treated groups and 2 control groups), levofloxacin was injected intraperitonialy in a doses of (37.5 mg/kg/day) and (75 mg/kg/day) and each dose was administered in two periods, short (14 days) and long duration (28 days). After completion of each duration of treatment, rats from each group were anesthetized using diethyl ether, blood and reproductive organ were collected from each rat for following parameters measurement.

Epididymal sperm preparation:

The caudal epididymis region of each rat was dissected, and located in 1 mL of pre-warmed Hams F10 medium (37^{0}C, 5% CO_2). Gentle tearing of the tissue was done to make spermatozoa swim out into the culture medium. The dishes were placed in the incubator for 15 min.

Microscopic examination:

The microscopic observation was done for each sample. A drop of sperm sample was added on a warm slide and covered with standard cover slip. The preparation was scored under light microscope (40 X) objective.

Sperm function parameters analysis

Certain sperm function parameters were examined namely; sperm concentration, motility, and morphology. Motility was calculated as the ratio of progressive motility including Rapid spermatozoa, Grade a; Slow spermatozoa, Grade b; non-progressive spermatozoa , Grade c; and immotile spermatozoa, Grade d [10] .

Sperm DNA integrity (Acridine Orange Test)

Acridine orange is a metachromatic fluorescence probe for estimation the degree of sperm nuclear DNA tendency for in-situ acid-induced denaturation by discrimination between native double-stranded DNA that resulting in green fluorescence and red fluorescent resulting from denatured single-stranded DNA [11]. Air-dried smears were fixed at room temperature in glacial acetic acid-methanol (1:3). The slides were detached from the fixative and leaved to dry, then stained with acridine orange (0.19 mg/mL, pH 2.5) at room temperature for 5 minutes. Solution of staining was daily formed from a stock solution composed of 1 mg acridine orange in 1 L of distilled water and put in the dark at 4^0C.

Aniline Blue (AB) staining

Aniline blue specifically stains histones rich in lysine and thus detecting anomalies in sperm chromatin condensation which are associated with residual histones [12]. In order to achieve this, air-dried smears from sperm samples were fixed with 3% buffered glutaraldehyde [in 0.2 M phosphate buffer (pH 7.2)] at room temperature for 30 min. Each smear sample was stained with 5% aqueous aniline blue in 4% acetic acid (7 min). Under light microscopic observation, 200 spermatozoa was counted in different areas of each slide using ×100 eyepiece magnification [13].

Histopathology of testes and epididymus

According to John D. Bancroft and Alan Stevens [14], histological examination of the tissues was performed following removal of organs from the rat.

Testosterone measurement

The serum was obtained through centrifugation of whole blood. Concentrations of testosterone in serum was obtained by radioimmunoassay using a readymade kit (ichroma boditech) for this purpose .

Statistical analysis

The study data were analyzed with SPSS software version 16 (SPSS, Inc., IL, USA). All results are presented as mean ± SE. Differences between quantitative data were analyzed with one-way ANOVA, followed by the Tukey test. P-Value less than 0.05 were considered significant for all data

RESULTS AND DISCUSSION:

Effects of levofloxacin administration on sperm concentration:

Administration of levofloxacin for 14 days results in a non significant reduction in sperm concentration while a significant reduction in sperm concentration was observed when levofloxacin was administered for 28 days and this reduction in the concentration was increase significantly as the dose of the administered levofloxacin was increased (table -1).

There are several postulated mechanisms by which sperm concentration can be adversely affected including direct toxicity of sperm or by inhibition of cell growth or cellular production [15], apoptosis in certain eukaryotic cells represent other mechanism and can be produced by interfering with the mitochondrial pathway [16], decrease testosterone level [17], in addition to decline in chromatin quality and /or DNA integrity can also have undesirable effect on sperm concentration [18]. In the present study testosterone level was not significantly affected by levofloxacin treatment, thus it cannot considered as a cause by which sperm concentration was adversely affected, but when chromatin quality and sperm DNA integrity were examined, our results indicate a marked elevation in the level of DNA fragmentation and reduction in sperm chromatin structure quality, therefore it can be concluded that these harmful effects on sperm genetic material can be considered as the mechanisms by which other sperm parameters such as sperm concentration being adversely affected in our present study.

Effects of levofloxacin on sperm motility:

No significant changes concerning the progressive and the total motility of sperm were resulted In the present study, when levofloxacin was administered for 14 days as compared with the control group, while there was a significant dose dependent reduction in the progressive motility and total motility when duration of treatment increased to 28 days (table 1). Physiologically, the sperm motility depends mainly on Ca^{++} influx and mitochondrial oxidation process to produce the energy required for hyperactivity and flagellum movement **[**19**].** Thus, the adverse effect of levofloxacin on sperm motility suggest that it may interfere with the function or the structure of Ca^{++} channels through direct or indirect toxicity. The other explanation is that, this agent may produce damage of CatSper channels. These channels are responsible for increasing the exhaustion of Ca ions which hyper activate sperm motility [20] .

Effects of levofloxacin administration on sperm morphology:

A significant increase in the percentage of morphologically abnormal sperm was seen when levofloxacin was administered in high dose for 28 days as shown in table (1). Evidences from preceding reports in sperm morphology indicated that, change in the morphology of sperm can be resulted from alterations in the compaction of chromatin [21, 22] . These postulated mechanisms for sperm morphological abnormalities are compatible with our findings since in the present study, it is founded that levofloxacin produce a significant harmful effects on sperm chromatin structure in the treated groups.

Effects of levofloxacin on sperm DNA integrity and chromatin quality:

Treatment with levofloxacine results in a significant increase in acridine orange staining capability of sperm DNA as compared with control groups, in addition there was a significant increase in capability of acridine orange staining as the dose and/or the duration of treatment with levofloxacin was increase as shown in table (2).

In addition, administration of levofloxacin for 14 days results in a significant increase in the percentage of positively stained sperm with aniline blue staining as compared with control group, with a significant increase in the susceptibly of aniline blue staining in correlation with the duration of treatment as shown in table (2). Our findings showed that following levofloxacin administration, the level of sperms with a single stranded DNA (sperms with DNA damaged which are positively stained with acridine orange) and immature sperms (sperms with impairment in protamine which are positively stained with aniline blue) were increased significantly. Many theories concerned with the abnormal chromatin packing like abnormal endogenous nicks in DNA and any break or dysfunction in the nucleases [23, 24]. The occurrence of high level of DNA nicks reflects cell requirement to unwind the torsional strain that resulted from negative supercoiling which, on the other hand, related with the displacement of protamines instead of nucleosomal histones and the modification of tertiary structure in elongating spermatids. As a result, the presence of DNA nicks in elongating spermatids reflecting a physiological necessity. These nicks are not risky as they are persistently ligated by topoisomerase II enzyme before completion of spermiogenesis. However, these nicks cannot be repaired suitably if abnormalities in topo II ligating activity are present or if this activity is blocked by the inhibitors of topo II enzyme [25]**.** Because levofloxacin is known as topoisomerase inhibitor [26]**,** so we can conclude that this drugs would block the creation and ligation of DNA nicks which in turn disturbs protamination and as a result can stimulate

internal damage in DNA by rising its propensity to damage and preventing its repair. This theory has been proofed with elevation of DNA damage as indicated with the positive acridine orange and aniline blue staining in sperms after levofloxacin administration in the current study. DNA damage can be considered as indicator of male subfertility [27].

Effect of levofloxacin on serum testosterone level

In the present study administration of levofloxacin in different doses and duration show no significant differences in the level of serum testosterone as compared to control groups (table -3), these findings were in agreement with Ahmadi, *et al.* (2016) [28].

Histopathology of testes, epididymus

When levofloxacin was administered for 14 days no significant changes concerning spermatogenesis was observed in the histological sections. The testes of rat treated with levofloxacin showed changes in the number of spermatozoa in some seminiferous tubules as there was a non-significant reduction in the number of spermatozoa inside them and some of epididymal tubules revealed reduction or absence of sperm specially when levofloxacin was administered in high doses and for 28 days ,these results are shown in figures (1)- figure (7). Similar findings were observed by Ahmadi, *et al.*, (2016), they had found that the administration of therapeutic doses of levofloxacin can produce pathological changes including atrophy in the seminiferous tubule with irreversible damages to testis cells which would resulted in interruption in the normal cycle of spermatogenesis [28]. Thus, high dose and long duration treatment confirm the theory that administration of some antibiotics such as levofloxacin, could disturb the process of spermatogenesis.

Table(1): Effects of intraperitonial administration of levofloxacin on sperm motility, concentration, and morphological normality in adult male rats. Values are expressed as mean ± standard error (n=8)

Treatment	Duration (days)	Progressive motility%	Total motility%	Immotile sperm%	Sperm concentration $^{x}10^{6}$ Sperm/ml	Morphologically normal sperm %
Control	14	49.00 ±1.41 (a)	80.71 ±0.92 (a)	19.29±0.92 (a)	40.14 ± 1.32 (a)	91.71 ± 0.42 (a)
	28	52.00 ±1.27 (a)	83.00 ±1.27 (a)	17.00±1.27 (a)	37.71 ± 1.61 (a)	92.57 ± 1.00 (a)
Levofloxacin (37.5 mg/kg/day)	14	45.86 ±1.28 (a)	80.29 ±1.17 (a)	19.71±1.17 (a)	39.14 ± 0.59 (a)	92.14 ± 1.06 (a)
	28	38.00 ±0.62 (b)	73.14 ±1.22 (bc)	26.86±1.22 (bc)	32.57 ± 0.81 (b)	89.57 ± 0.87 (ab)
Levofloxacin (75 mg/kg/day)	14	44.57 ±0.97 (a)	78.71±1.01 (ab)	21.29±1.02 (ab)	36.00 ± 0.62 (a)	91.85 ± 1.34 (a)
	28	31.14 ±0.91 (c)	70.29 ±2.26 (c)	29.71±2.26 (c)	28.00 ± 0.49 (c)	87.57 ± 0.90 (b)

Values on the same column carrying the same letter are not significantly different.

Table (2): Effects of intraperitonial administration of levofloxacin on sperm DNA integrity and chromatin quality in adult male rats. Values are expressed as mean ± standard error (n=7)

Treatment	Duration (days)	Positive acridine orange staining %	Positive aniline blue staining %
Control	14	7.57 ± 0.37 (a)	9.71 ±0.57 (a)
	28	6.29 ± 0.42 (a)	10.14 ±0.94 (a)
levofloxacin (37.5 mg /kg/day)	14	27.43 ± 0.72 (b)	16.14 ±0.77 (b)
	28	46.14 ± 0.55 (d)	21.14 ±1.22 (c)
levofloxacin (75mg/kg/day)	14	36.00 ± 0.76 (c)	17.43 ±0.69 (b)
	28	58.29 ± 0.75 (e)	23.14 ±0.67 (c)

Values carrying the same letters are not significantly different .

Table (3): Effects of intraperitonial administration of levofloxacin on serum testosterone level in adult male rats. Values are expressed as mean ± standard error (n=7)

Serum Testosterone level		
Treatment	**14 days**	**28 days**
Control	1.79 ± 0.29 (a)	1.93 ± 0.27 (a)
levofloxacin (37.5 mg/kg/day)	1.86 ± 0.17 (a)	2.32 ± 0.33 (a)
levofloxacin (75 mg/kg/day)	2.33 ± 0.27 (a)	2.48 ± 0.22 (a)

Values on the same column carrying the same letters are not significantly different .

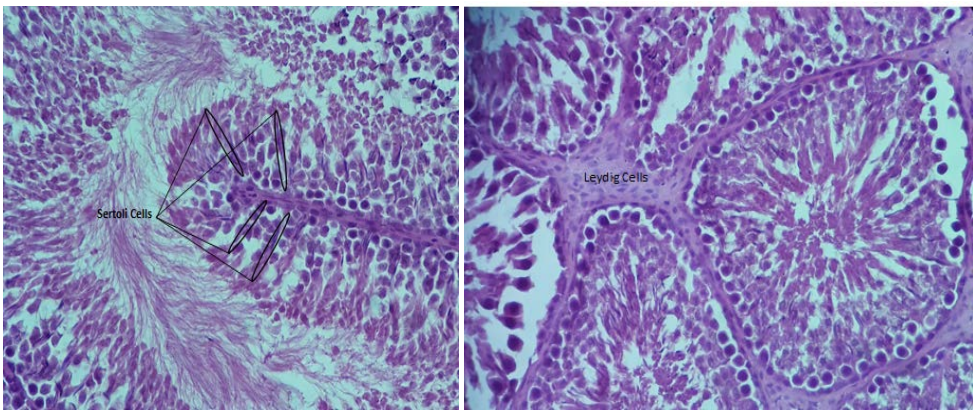
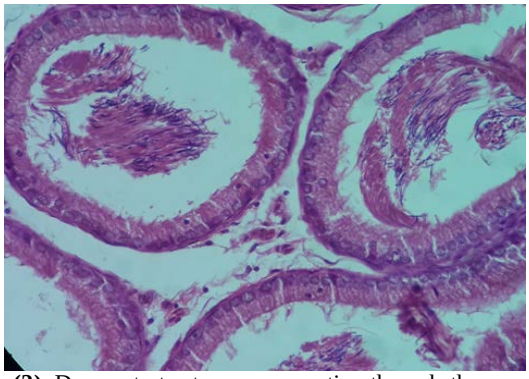


Figure (1): Demonstrates transverse section through the seminiferous tubules of control rat showing regular spermatogenesis reaching the level of spermatozoa. H&E. (X400).



Figure(2): Demonstrates transverse section through the epididymis of control rat studded with spermatozoa. H&E. X400.

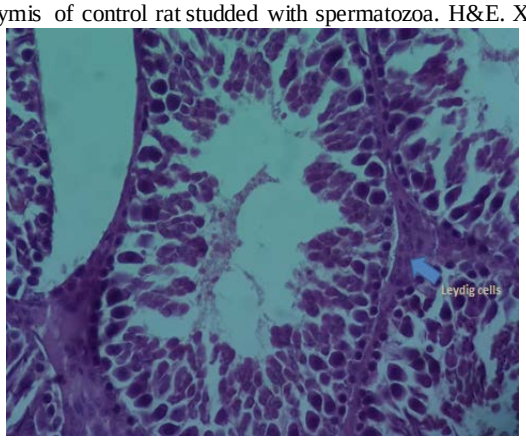


Figure (3): Demonstrates transverse section through the seminiferous tubules of Levofloxacin- treated rat (75 mg/kg/day for 28 days). H&E. X400.

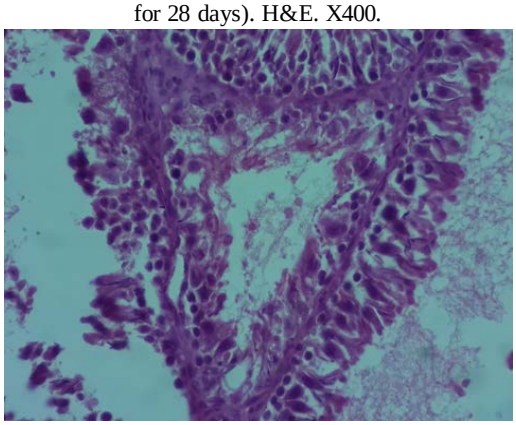


Figure (4): Demonstrates transverse section through the seminiferous tubules of Levofloxacin- treated rat (37.5mg/kg/day for 28 days). H&E. X400.

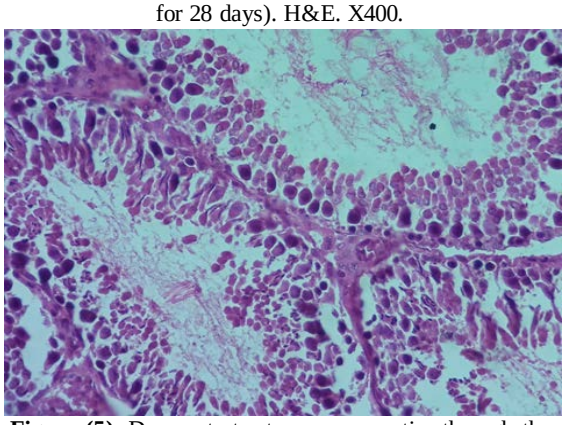


Figure (5): Demonstrates transverse section through the seminiferous tubules of Levofloxacin- treated rat (75 mg/kg/day for 14 days). H&E. X400.

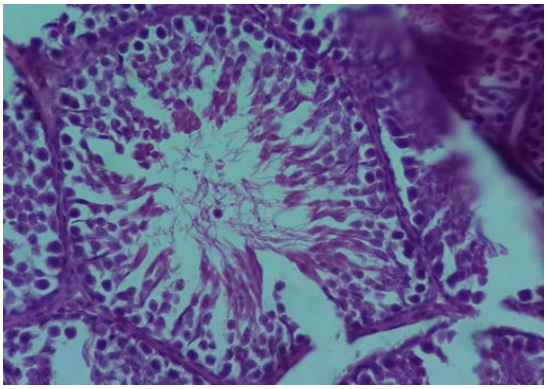


Figure (6): Demonstrates transverse section through the seminiferous tubules of Levofloxacin- treated rat (37.5 mg/kg/day for 14 day). H&E. X400.

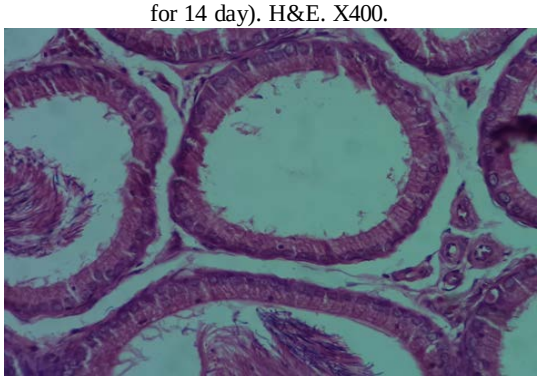


Figure (7): Demonstrates transverse section through the epididymis of Levofloxacin- treated rat. H&E. X400.

CONCLUSION:

Levofloxacin could adversely affect the process of spermatogenesis in rats in a time and dose dependent manner .

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