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RESEARCH ARTICLE

Impacts administration of Rifampicin on sperm DNA integrity and Male Reproductive System parameters in rats

Dr. Furqan Mohammed Al-Asady[1], Dr. Dalia Abdulzahra Al-Saray[2]
[1]College of Pharmacy, University of Al-Ameed, Karbala Po Box 198, Iraq.
[2]College of Dentistry, Babylon University, Department of Basic Sciences, Iraq.
*Corresponding Author E-mail: **dr.furqan.alasady@gmail.com**

ABSTRACT:

Objective: Evaluate the impacts of rifampicin on certain sperm function parameters and to determine whether rifampicin has an impact on chromatin quality or sperm DNA integrity. **Materials and Methods:** Forty two male adult rats were subjected to this study. The entire rats were subjected to random division into six groups; four rifampicin- treated groups and two control groups. Rifampicin- treated groups were treated with a dose of either (27mg/kg/day) or (54mg/kg/day) and for each treatment dose, the treatment persists for either 14 days or 28 days. Certain parameters of sperm function including sperm concentration and sperm motility were assessed. Furthermore, analysis of sperm DNA integrity and chromatin quality were also studied. **Results:** No significant changes related to sperm concentration were observed when rifampicin was given in different doses and different durations. A significant change in sperm motility were recorded only when rifampicin was given in high dose for 28 days and there was a significant reduction in sperm progressive and total motility. Rifampicin showed a significant increase in sperm DNA staining capability when the dose and duration was increased. Administration of rifampicin in high dosage for 28 days represented in larger adverse impact on structure of sperm chromatin. **Conclusion:** Rifampicin could negatively affect male fertility potential in rats mainly through affecting the quality of sperm chromatin structure.

KEYWORDS: Rifampicin, Reproductive system, Male, Rats.

INTRODUCTION:

Abnormalities in sperm count are considered as the most important parameters in semen analysis, ranging from oligospermia (fewer than normal sperm) to azoospermia (undetectable sperm)[1]. Some pharmaceutical medications and recreational drugs have been estimated to affect stages of semen production and some medications are documented to decrease libido, as well as, impair erectile function and ejaculation[2]. Researches had reported that some antibiotics adversely affect the functional integrity of mature sperm and impair sperm motility features. Perfloxacin was estimated to have toxic effects on animal testicular function[3].

Rifampicin can be classified as related macrocyclic antibiotics that are produced by *Amycolatopsis mediterrane*; rifampicin represents a semisynthetic derivative of rifamycin B. Rifampicin act by suppression of initiation of RNA synthesis through inhibition of DNA-dependent RNA polymerase activity in susceptible cells. It can interacts with bacterial RNA polymerase without affecting the mammalian enzyme[4]. It acts through binding to the β subunit of DNA-dependent RNA polymerase (*rpoB*) to produce a stable drug-enzyme complex and as consequence, chain formation in RNA synthesis will be suppressed[5].

Rifampicin is active against coagulase-negative staphylococci and *Staphylococcus aureus.* The drug also has high activity against *Haemophilus influenzae* and *Neisseria meningitides*. Rifampicin is reported to inhibit the growth of *Legionella* species in animal models and in cell culture[6]. The most serious adverse reactions that is associated with rifampicin is hepatotoxicity, common side effects include rashes, fever, immunological reactions and gastrointestinal disturbances. Rifampicin

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administration usually resulted in a benign side effect represented by orange-red discoloration of certain body fluids, such as sweat, urine, and tears[7].

Also, rifampicin (which is regarded as cytochrome inducer) has been reported to have a role in the development of diabetic cataract through the induction of cytochrome enzyme which can lead by a special mechanism to accumulate the polyols within the lens that considered as an important contributing factor in diabetic cataractogenesis[8]. In addition to that, the hepatotoxicity which is most commonly caused by rifampicin was recorded to be significantly lowered in pretreatment with aqueous leaves extract of *Saccharum officinarum*[9].

MATERIALS AND METHODS:

Experimental animals:

Forty two adult male Albino-rats of the Wistar strain were used in the study. Their age was in range of 8-10 weeks old and the weight of these rats was ranged from 200-250gm. The rats were accommodated in cages with light-dark cycles in range of (11-14) hours light-dark cycles under controlled temperature (nearly 25°C) and were fed standard commercial pellets. Following adaptation, these rats were randomly divided into six groups (7 rats in each group). The groups involved four rifampicin groups and two control groups, rifampicin was injected intraperitonialy in a dose of (27mg/kg/day) and (54mg/kg/day); each dose was given in two durations (short for 14 days and long for 28 days). At the end of each treatment period, rats from each of the six groups were anesthetized with diethyl ether. After that, blood and reproductive organs from each rat were collected for further investigation in the study. The work on the animal was approved by ethics committee in Iraq which is the Institutional Review board (IRB).

Preparation of Epididymal sperm:

The caudal region of epididymis of each rat was recovered and put in 1ml of Hams F10 medium (previously warmed, 37ºC, 5% CO_2). Ripping of tissue gently with to ensure spermatozoa swim out into the culture medium. After that, dishes were put for 15 minutes in the incubator.

Microscopic examination:

For each sample in the study, the microscopic observation was made. 10µl of sperm sample was put on a pre- warmed slide then covered with standard cover slip and the preparation was recorded under light microscope (40 X objective).

Analysis of Sperm Function Parameters:

Some sperm function parameters were analyzed including sperm concentration and sperm motility. Motility was represented by the percentages of progressive motility and this was expressed by rapid spermatozoa (Grade A) and slow spermatozoa (Grade B), non-progressive spermatozoa (Grade C) and the immotile spermatozoa (Grade D) spermatozoa[10].

Sperm Concentration:

Sperm concentrations were calculated from the mean number of sperm in five high power fields under magnification of 40 X. This number was multiplied by factor of one million[11].

Sperm concentration (million/ml) = number of sperm/ HPF × 10^6

Sperm Motility:

Under microscope, the prepared slide was observed for sperm motility detection. It was examined instantly to prevent the negative effect of heat of microscope light source which affect sperm motility[12]. The number of motile sperm in five randomly selected fields was counted away from the cover slip edge and at least one hundred spermatozoa were counted. One hundred spermatozoa on plain slide was observed and the number of progressively motile and immotile sperm was then recorded. Sperm were scored in four categories according to WHO[10]:

Rapid (Grade A) and Slow spermatozoa (Grade B), non-progressive (Grade C) and immotile spermatozoa (Grade D).

Percentage motility of sperm was counted by the following formula:

$$\text{Total sperm motility percentage} = \frac{\text{No. of motile sperm}}{\text{Total No. of sperms}} \times 100$$

Evaluation of Sperm DNA Integrity using Acridine Orange:

A metachromatic fluorescence probe (acridine orange) is utilized for evaluation the grade of sperm nuclear DNA inclination for in-situ denaturation by acid in order to discriminate between native double-stranded DNA which resulted in green fluorescence and denatured single-stranded DNA that associated with red fluorescence[13]. An overnight fixation of smears (air-dried) at room temperature in methanol- glacial acetic acid in a ratio of (1:3) was performed. The fixative was removed from slides and put to dry, then acridine orange was applied for 5 minutes (pH 2.5, 0.19mg/mL) at room temperature. Staining solution was daily prepared from a stock solution included one mg of acridine orange dissolved in one Litter of distilled water and stored at 4^0C in the dark.

Evaluation of sperm chromatin quality using aniline blue (AB) staining:

Lysine-rich histones are selectively stained by aniline blue. Thus it has the ability to show those sperm chromatin condensation anomalies which are associated with residual histones. To do this staining, air-dried smears was primarily fixed in 3% buffered glutaraldehyde in 0.2 M phosphate buffer of 7.2 pH and samples were remain in the fixative agent for 30 minutes at room temperature. After fixation, each smear was stained with 5% aqueous aniline blue stain [in 4% acetic acid (pH=3.5)] for 7 min. In light microscopic evaluation, 200 spermatozoa were evaluated in different areas of each slide using ×100 eyepiece magnification[14].

Statistical analysis:

The data of the study were analyzed with SPSS software version 16. All results parameters are presented as mean ±SE. Differences between quantitative data were analyzed with one-way ANOVA, followed by the Tukey test. P-Values less than 0.05 were considered significant for all data[15].

RESULTS:

Rifampicin administration effects on concentration of sperm:

No significant changes (p>0.05) regarding concentration of sperm was noticed as rifampicin was administered in various durations and various doses in the current study when compared with control groups as illustrated in (table-1).

Table 1: Effects of intraperitonial administration of rifampicin on sperm concentration at different durations in adult male rats.

Treatment	Sperm concentration (sperm/ml)	
	14 days	28 days
Control	40.14 ± 1.32 $^{x}10^{6}$ (a)	37.7 ± 1.6 $^{x}10^{6}$ (a)
rifampicin (27mg/kg/day)	36.85 ± 1.4$^{x}10^{6}$ (a)	35.28 ± 0.9$^{x}10^{6}$ (a)
rifampicin (54mg/kg/day)	36.00 ± 1.82$^{x}10^{6}$ (a)	34 ±1.5$^{x}10^{6}$ (a)

Values on the same column that carry the same letter are not significantly different (p>0.05). Values are expressed as mean ± standard error (n=7)

Rifampicin administration effects on motility of sperm:

No significant alterations (p >0.05) in motility of sperm with rifampicin administration were noticed except when it was administered in high dose for 28 days, a significant reduction (p<0.05) in sperm progressive motility and sperm total motility as compared with control group and best shown in (table 2).

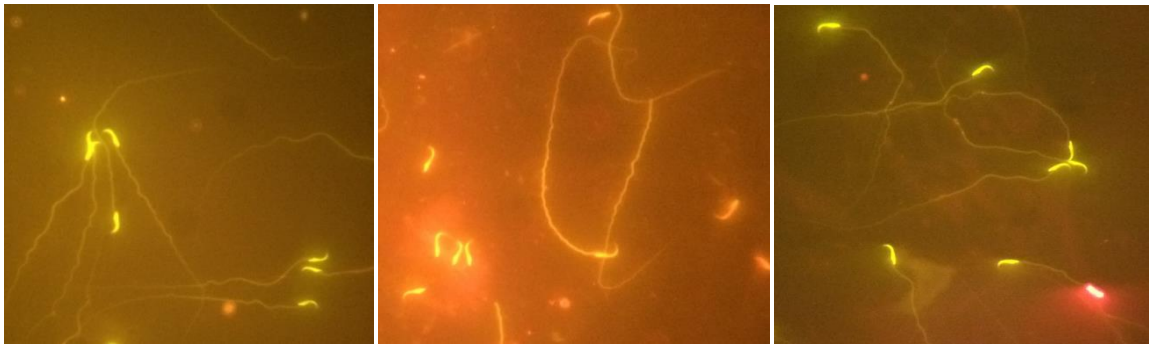
Effects of rifampicin treatment on DNA integrity of sperm:

When rifampicin was given in short duration and low dose, no significant alterations (p > 0.05) in sperm DNA staining capability (with acridine orange stain) was seen. On the other hand, there was a significant rise (p < 0.05) in this staining ability (susceptibility of sperm DNA for denaturation) when both duration and dose of rifampicin were increased as summarized in (figure 1) and (table 3).

Table 2: Effects of intraperitonial administration of rifampicin on sperm motility in adult male rats.

Treatment	Duration	Total motility%	Progressive motility%	Immotile sperm %
Control	14 days	80.71 ±0.918 (a)	49.00±1.41 (a)	19.29±0.92 (a)
	28 days	83.00 ±1.27 (a)	52.00±1.27 (a)	17.00±1.27 (a)
rifampicin (27mg/kg/day)	14 days	78.86 ±1.99 (a)	46.29±2.16 (a)	21.14±1.99 (a)
	28 days	74.71 ±2.75 (a)	43.14±2.34 (a)(b)	25.29±2.75 (a)
rifampicin (54mg/kg/day)	14 days	79.86 ±2.51 (a)	47.29±2.34 (a)	20.14±.2.51 (a)
	28days	73.57 ±1.73 (b)	38.29±0.87 (b)	26.43±1.73 (b)

Values on the same column which carry the same letter are not significantly different (p >0.05). Values are expressed as mean ± standard error (n=7)



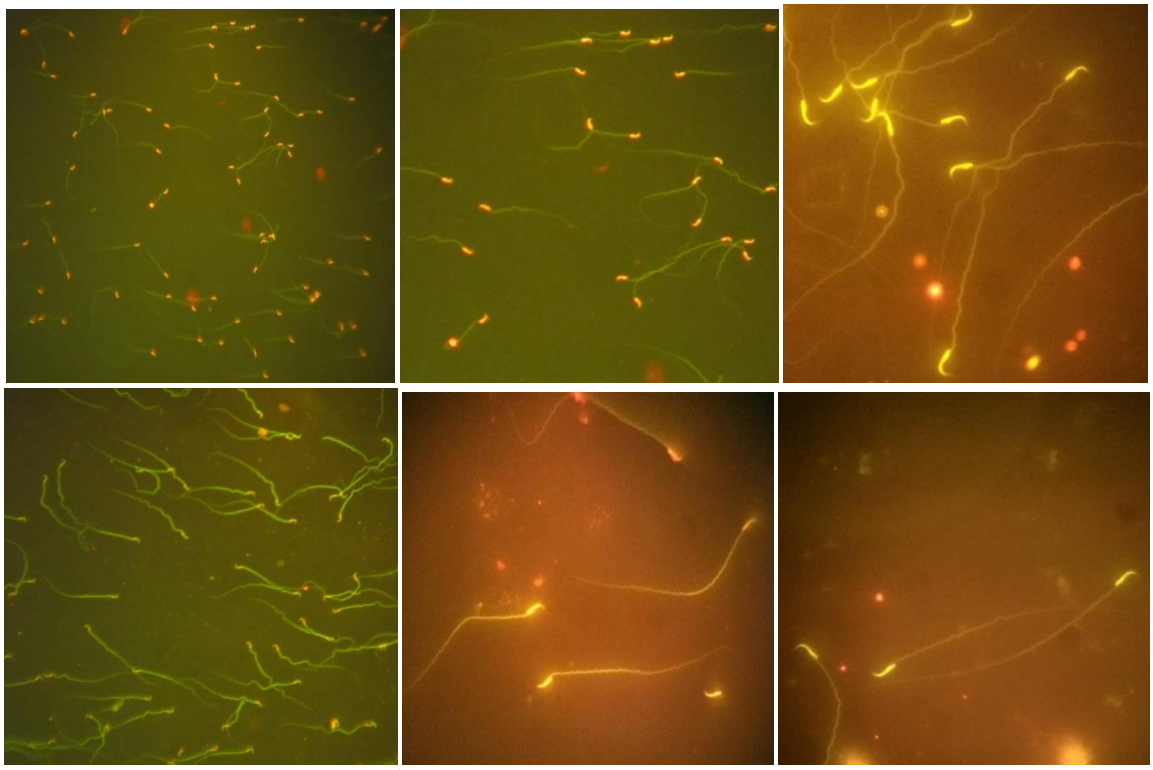


Figure 1: Fluorescence microscopic images of acridine orange de-stained cells which shows that spermatozoa producing green fluorescence are considered to have normal DNA content, whereas sperms that produce a spectrum of yellow-orange to red fluorescence are considered to have damaged DNA.

Table 3: Effects of intraperitonial administration of rifampicin on susceptibility of sperm DNA denaturation in adult male rats.

Treatment	**Positive acridine orange staining %**	
	14 days	**28 days**
Control	7.57 ± 0.37 (a)	6.29 ± 0.42 (a)
rifampicin (27mg/kg/day)	11.29 ± 1.19 (a)	11.71 ± 1.46 (a)(b)
rifampicin (54mg/kg/day)	10.86 ± 1.55 (a)	17.00 ± 1.99 (b)

Values on the same column that carrying the same letters are not significantly different ($p > 0.05$). Values are expressed as mean ± standard error (n=7)

Effects of rifampicin treatment on chromatin quality

Rifampicin administration yield a significant increase ($p < 0.05$) in percentage of sperm with positive aniline blue staining (percentage of affected chromatin structure of sperm) as compared with the control group and this result was significantly ($p < 0.05$) linked with period and dose of rifampicin treatment as illustrated in (table 4) and (figure 2).

Table 4: The effect of intraperitonial administration of rifampicin on chromatin quality in adult male rats

Treatment	**Positive aniline blue staining %**	
	14 days	**28 days**
Control	9.71 ±0.57 (a)	10.14 ±0.94 (a)
rifampicin (27mg/kg/day)	19.29 ±0.92 (b)	35.00 ±1.22 (d)
rifampicin (54mg/kg/day)	27.29 ±3.42 (c)	45.71 ± 1.87 (e)

Values that display the same letters are not significantly different ($p > 0.05$). Values are expressed as mean ± standard error (n=7)

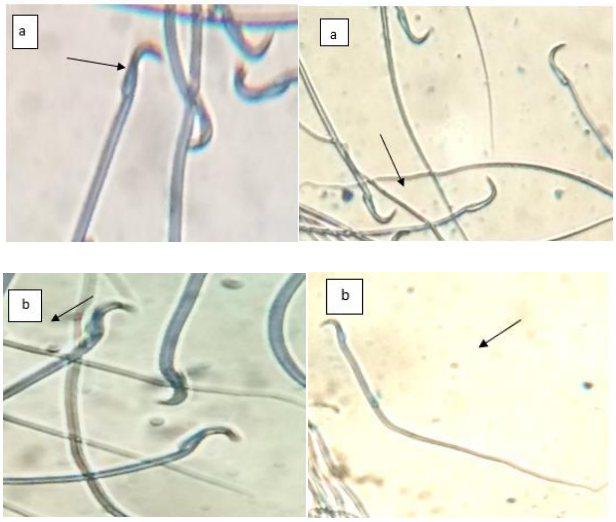


Figure (2): Sperm chromatin condensation assessed by aniline blue.
(a) Sample showing mainly mature sperm with unstained nucleus.
(b) Sample showing mainly immature sperm with blue-stained nucleus.

DISCUSSION:

Duration of rifampicin administration in the present study with gave no effect on sperm concentration and was incompatible with Awodele *et al.*, (2010)[16] who found that administration of rifampicin in a dosage of (9mg/kg/day) for 90 days resulted in a significant decrease in sperm count. These variations in results

might be due to difference in treatment period because Awodele *et al*., gave rifampicin for a period of 90 days as compared with 28 days which was the longest period of drug administration in this study.

Many assumed mechanisms could explain this adverse effect by these drugs include suppression of cell growth or cellular production sperm cell direct toxicity[17], interfering with mitochondrial pathway represents other mechanism that can lead to apoptosis in certain eukaryotic cells[18].

Rifampicin has the ability to increase the motivation of cytochrome P-450 2E1. This enzyme could be able to generate reactive oxygen species, like superoxide radicals, and as a result, fast reaction between organic molecules and these intermediates could be produced, initiating secondary free radicals species. Such mechanism could resulted in a condition of sperm oxidative damage and errors in spermiogenesis (abnormal spermatozoa)[19].

Physiologically, the motility of sperm relies primarily on influx of Ca^{++} and process of mitochondrial oxidation to generate energy necessary for hyperactivity and movement of flagellum[20]. Therefore, the adverse effects of rifampicin on motility of sperm propose that this agent may interfere with the structure or function of Ca^{++} channels and these effects were time and dose dependent.

It had been previously assumed that rifampicin has direct destructive impacts on DNA and could be attributed to free radical production by rifampicin which could finalized by lipid peroxidation of membrane causing DNA fragmentation and subsequent covalent binding between sperm DNA and product of lipid peroxidation. Whereas Other studies indicated the presence of highly-inducible isoform of cytochrome P-450 2E1 in male gonads and because this cytochrome can be stimulated by rifampicin and can produce reactive oxygen intermediates, so rapid reaction with organic molecules happen producing secondary free radicals. Such cascades can resulting in a conditions of sperm oxidative damage [21].

The yields of present work revealed that administration of rifampicin in high dosage for 28 days represented in larger adverse impact on structure of sperm chromatin. This effect might be obtained from its behavior by inhibition of DNA-dependent RNA polymerase (as a result inhibition of RNA synthesis)[22]. Because this enzyme is associated with protein synthesis, it can be assumed that as high dose of rifampicin was given, drug could lose its selectivity toward bacterial enzyme, and directing mammalian one. When enzyme activity has been abolished, protein synthesis will be negatively affected, thus, protein content of chromatin will be changed making it more vulnerable to interact with acidic dyes (as aniline blue) which is confirmed by the present results (figure 2).

CONCLUSION:

Rifampicin antibiotic could initiate adverse impacts on fertility potential in male rat and this negative effects of rifampicin on male fertility is a dose- and duration-dependent manner. Rifampicin exerts its adverse effects on male fertility mainly through affecting the quality of sperm chromatin structure.

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CONFLICT OF INTEREST:

No conflict of interest.

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