RESEARCH ARTICLE

Effect of Azithromycin on Sperm DNA of Male Rats

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ABSTRACT

Background: Infertility is a psychological, economic, and medical problem that involves trauma and stress, with a strong emphasis on child-bearing. Antibacterial agents are used to treat a wide range of infections, cancer, protozoa, and helminths. However, antimicrobial therapy has been shown to significantly impact human and animal models sperm parameters Azithromycin is a new 15-membered ring azalide antibiotic that exhibits enhanced *in vivo* activity against local infections due to its enhanced efficacy against gram-negative bacteria and high persistent tissue levels. This study was designed to investigate the possible fertility (Azithromycin) side effects of male rats by assessing the DNA fragmentation of sperm.

Methods: This study was performed on 24 grown-up Wistar pale-skinned adult rats. It matured for 11 to12 weeks and weighed (180–310) grams, according to the guidelines of the Biochemistry and Research Ethics Committee, approved by the Scientific Committee of the Center for Biotechnology Research, University of Al-Nahrain. The study was carried out from January 2021 to March 2021. The animals were sourced from the Animal Facility of the University of Al-Nahrain Biotechnology Center. It is then divided into three groups (8 animals each). They are housed in a highly ventilated room in a plastic housing and are fed on a standard pellet diet, and drinking water is not obligatory during the test. All animals were bred under standard laboratory conditions with a temperature of 25 ± 2°C and a 12 hours light and dark cycle. Azithromycin, a subclass of macrolide antibacterial, is marketed by pharmaceutical company Pfizer as (Zithromax®) in suspension dosage form as azithromycin dihydrate powder. Azithromycin was orally administered by gavage tube twice every day to rats (180–31) body weight with a dose of 45 mg/kg body weight (treatment dose) and 90 mg/kg body weight (double-treatment dose).

Results: In concepts of DNA damage levels in testes, among the three groups (Azithromycin, both therapeutic & double therapeutic doses and control) and by the utilization of a one-way ANOVA test, the results indicate that DNA damage in testes was a significant effect ($p < 0.05$) in animals treated with all doses of Azithromycin & each compared with the control animals.

Conclusion: DNA damage in testes by comet assay showed there was a significant effect ($p < 0.05$) in animals treated with all doses of Azithromycin each compared with the control animals in different degrees (low, medium, high) damage.

Keywords: Azithromycin, Infertility, Male rat, Oxidative stress, Sperm count.

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INTRODUCTION

Infertility is a reproductive system disease defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, according to the World Health Organization's International Committee for Monitoring Assisted Reproductive Technology (WHOICMAFT).[1,2]

Oxidative stress is defined as a condition caused by an imbalance in the production and accumulation of reactive oxygen species (ROS) in cells and tissues, as well as a biological system's ability to detoxify these reactive products.[3] Superoxide radicals (O_2•), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen are all examples of reactive oxygen species (ROS) ($^1O_2$). Mitochondria primarily produce ROS in both physiological and pathological conditions; for example, O_2• can be formed by cellular respiration, lipoxygenases (LOX), and cyclooxygenases (COX) during arachidonic acid metabolism and endothelial and inflammatory cells.[4]

To protect themselves from ROS-induced cellular damage, cells employ an antioxidant defense system that is primarily comprised of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) are enzymatic components (GPx).[5]

ROS production had been shown in studies to be directly related to testosterone and androstenedione levels.[6]

The glutathione protein family, superoxide dismutase, catalase, and several non-enzymatic antioxidants all aid the

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testes in mitigating the toxicity.[7] ROS in sperm is produced by both sperm cells and infiltrating leukocytes.[8]

Another method, the comet assay, which is used to recognize DNA damage in cells,[9] is one example. The redox couples GSH/GSSG and cysteine/cysteine in plasma have been studied, and high cystine levels have been associated with death in cardiovascular disease.[10]

Comet assay, a simple and direct method for detecting DNA breaks, cells are embedded in agarose, lysed, and electrophoresed at high pH; broken DNA is drawn to the anode, forming a comet-like image when observed under fluorescence microscopy.[9] The comet assay has developed into the most widely used method for measuring ROS.

MATERIALS AND METHODS

Azithromycin, a subclass of macrolide antibacterial, is marketed by pharmaceutical company Pfizer as (Zithromax®) in suspension dosage form as azithromycin dihydrate powder. Azithromycin was orally administered by gavage tube twice every day to rats (180–310 body weight) with a dose of 45 mg/kg body weight (treatment dose) and 90 mg/kg body weight (double-treatment dose).

The antibiotic doses were selected based on the rat guide.[11,12] Azithromycin was prepared by adding sterile water to the bottle up to 15 mL to get a suspension with a concentration of (200 mg/5 mL) and then administered to the animal twice daily.

This prospective study was performed on 24 grown-up Wistar pale-skinned adult rats. It matured 11–12 weeks and weighed (180–310 g). After three days of adaptation, experimental rats were randomly directed to one of There are three main classes of eight rats each, as follows:

For two weeks, rats received distilled water (DW) orally via gavage tube. This group served as the negative control group.

For 14 days, rats in Group B were provided a treatment dose of azithromycin dihydrate solution (45 mg/kg/day) orally via a gavage tube.

Group C: For 14 days, rats received a double treatment dose of azithromycin dihydrate solution (90 mg/kg/day) orally via a gavage tube.

The dose had been given daily according to the animal's body weight. The drug doses were adjusted daily according to the animal's mean body weights.

Rats were euthanized with diethyl ether, then cervical dislocation in 21 days after the end of the procedure (i.e., on day 35). The testes were excised, measured, and washed with 0.9% normal saline. For histopathological examinations, testes were fixed in 10% neutral buffered formalin.

Individual cell DNA damage was evaluated using a commercial kit, the OxiSelect™ Comet Assay kit (Cell Biolabs, Inc., USA), in accordance with Singh *et al*.[13] methods (Singh *et al*., 1988). The Comet assay is a single cell gel electrophoresis (SCGE) assay used to assess cellular DNA damage. Before being applied to the OxiSelectTM Comet Slide, individual cells are mixed with molten agarose. The embedded cells are then treated with a lys. Finally, electrophoresis in a horizontal chamber differentiates the specimens into intact and damaged DNA fragments. After electrophoresis, the specimens are dried, stained with a DNA dye, and investigated under epifluorescence microscopy. Damaged DNA (with cleavage and strand breaks) will relocate further than intact DNA under these circumstances, resulting in a "comet tail" form is buffer. Furthermore, an alkaline solution relaxes and denatures the DNA.

Statistical Analysis

Mini Tab was used to perform statistical analysis on the data (Software package for statistical analysis - version 18). Mean, and standard deviation (mean ± SD) were used to express descriptive statistics for numerical data. To assess the significance of differences between groups, one-way and two-way analysis of variance (ANOVA) and the least significant difference posthoc test were used. $p < 0.05$ is regarded as statistically significant.

Statistical Analysis

Mini Tab was used to perform statistical analysis on the data (Software package for statistical analysis - version 18). Mean, and standard deviation (mean ± SD) were used to express descriptive statistics for numerical data.

RESULTS

In concepts of DNA damage levels in testes, among the three groups (Azithromycin both therapeutic and double therapeutic doses and control) and by the utilization of one-way ANOVA test, the results in (Table 1) and (Figures 1–3) indicate that DNA damage in testes was significant effect ($p < 0.05$) in animals

Table 1: Distribution of DNA damage levels (mean ± SD) by Comet assay

Parameters groups	*No damage % Mean + SD*	*Low damage % Mean + SD*	*Medium damage % Mean + SD*	*High damage % Mean + SD*
Control	A 44.825 + 1.274	A 44.069 + 1.293	A 5.474 + 1.088	A 5.631 + 0.783
Azithromycin	C 40.545 + 1.403	B 40.414 + 1.073	B 9.591 + 1.418	B 9.451 + 0.982
Azithromycin double	D 36.547 + 1.432	C 38.157 + 0.612	D 12.153 + 1.385	D 13.143 + 0.702
p-value	0.00012	0.00024	0.0001	0.00032
LSD	1.854481	1.761403	1.754433	1.347266

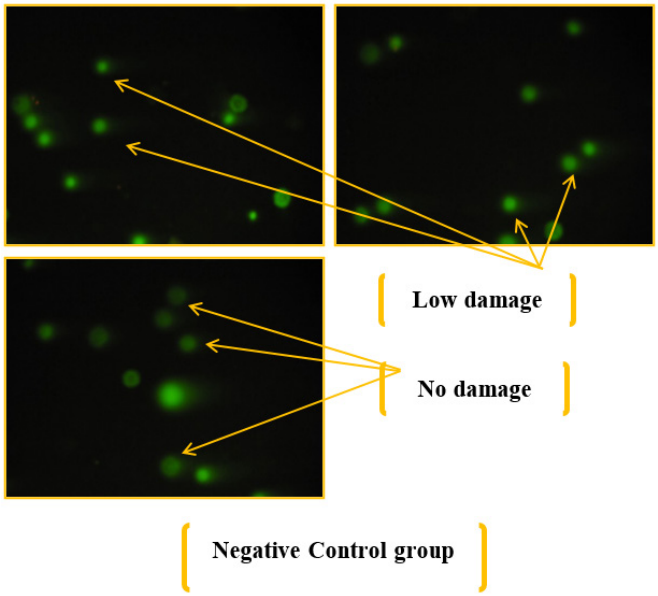

Figure 1: Types of DNA damage as observed in (Negative Control group)

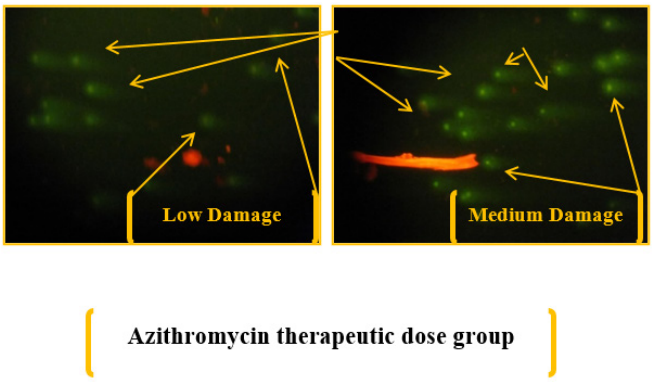

Figure 2: Types of DNA damage as observed of treated animals (azithromycin therapeutic dose treated group) examined by fluorescent microscope

treated with all doses of azithromycin each compared with the control animal.

DISCUSSION

Unexplained infertility is a challenging task for healthcare professionals to alleviate, but it is a frustrating experience for married people. The routine sperm analysis immediately creates an entirely normal report, but there is no method of determining whether the functional property of sperm is normal or not. The kind of chemotherapeutic agent used, the dosages and length of treatment, and the testis' stage of development all affect the testicular damage's severity.[14]

Indeed, the drug's ionization and lipid hydrophilicity enable it to enter the male genital tract via an ion-trapping system.[15,16]

Based on the measurements obtained from a standard sperm analysis, sperm with DNA damage may be recognized as a distinct marker of male infertility. Furthermore, sperm samples from infertile men with normal sperm parameters

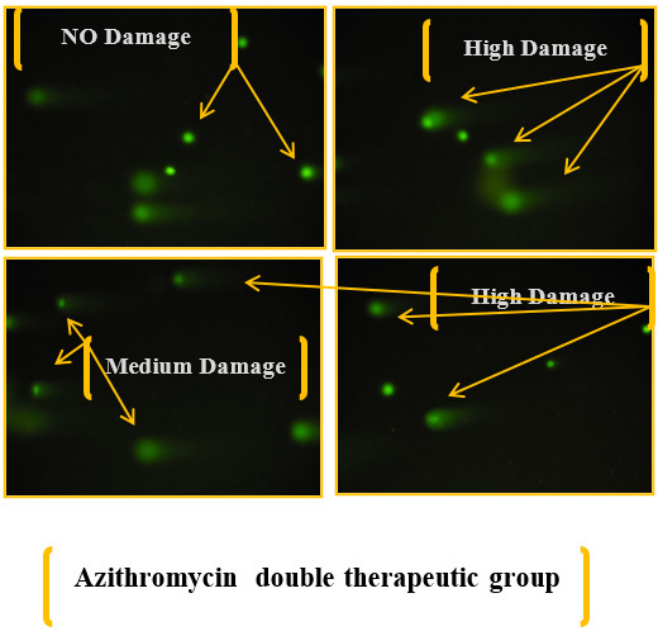

Figure 3: Types of DNA damage as observed of treated animals (Azithromycin double therapeutic dose treated group)

showed increased DNA damage. More than 30% of sperm DNA fragmentation may have a negative effect on male reproductivity.[17,18]

The comet test is a well-known responsive method for detecting sperm DNA damage by identifying broken DNA strands in various cell types.[17,19] It can detect the genotoxic potential of drugs and their metabolites by interacting with their genetic material.

Because sperm function tests alone were insufficient for determining all germ cell toxicants, the tail moment percent was the most incorporated parameter for assessing the cell's overall DNA damage.[20]

Chromatin handling and isolation errors,16 oxidative stress, abnormal cell apoptosis, and hormonal deficiencies[20,21] could cause sperm DNA damage. In studies assessing the relationship between sperm characteristics and DNA damage, there was no significant relationship between abnormal morphology and sperm DNA damage in men.[22,23] In another study, human sperms with typical morphologies but from infertile subjects were identified to have DNA fragmentation.[24]

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