DOI Number: 10.37506/v14/i1/2020/ijfmt/193013

Study Genotoxicity of Ciprofloxacin in white rats

Mustafa Ahmed Shihab[1], WAJDI Sabeeh Sadek[1]

[1]Biology department, College of Science, Tikirit University, Tikrit, Iraq

Abstract

The genotoxic and cytotoxic effects of ciprofloxacin drug on the bone marrow of white male rats were assessed by the Micronucleus test (MN) in polychromatic Erythrocytes (PCEs). The DNA damage was assessed by the Comet assay technique in bone marrow, liver and spleen. The white male rats were administered by oral gavage of single doses (93.5), (187) mg.kg^{-1} of body weight with twice a day for 7 consecutive days, while the negative control group was administered distilled water. The results showed a significant increase in the number of micronuclei corresponding to the duration of exposure., there was a change in the percentage of immature erythrocytes in bone marrow. This increased with an increase in treatment duration. As for the number of immature erythrocytes containing micronuclei, there was a significant increase corresponding to the treatment duration. When using the comet assay technique, the values of DNA damage increased in a dose-related manner.

Keywords : *Ciprofloxacin, Comet Assay, Micronucleus.*

Introduction

Ciprofloxacin (CFX) is a broad spectrum antibiotic belonging to the fluoroquinolone family [1]. It is regarded as the second generation of this family and has a wide spectrum and efficacy compared to other types within this family. Ciprofloxacin is considered the most commonly known antibiotic, used to treat many bacterial infections in the respiratory tract, skin, bones, joints, urinary tract infections and diarrhea [2].

It is an antibiotic that kills bacteria by altering the efficiency and function of the DNA gyrase enzyme, which is responsible for bacterial DNA vas damage. This inhibition of the DNA gyrase effeciency causes rapid bacterial death [3, 4].

Test design:

5 rats for MMC positive control, 5 rats for negative control distilled water, 5 rats for each treatment with doses of 93.5 and 187 mg.kg^{-1} of body weight.

Micronucleus Assay of immature erythrocytes in the male bone marrow of white male rats.

Corresponding author:
WAJDI Sabeeh Sadek
E-mail: marwan.walady@yahoo.com

The animals were sacrificed by separating the cervical vertebrae after 18 hours of gavage for negative and positive control groups and those treated with ciprofloxacin, and the test was performed in the manner described by Schmid [5].

Estimation of DNA damage in bone marrow, liver and spleen cells in male white rats using a comet assay technique:

Animals were sacrificed by cervical vertebral separation after 24 hours of gavage administration, and the test was performed according to the protocol described by Dhawan *et al* (2001) [6].

Results and Discussion

Studying the effect of ciprofloxacin drug (CFX) in inducing the formation of micronuclei (MN) in white male rats.

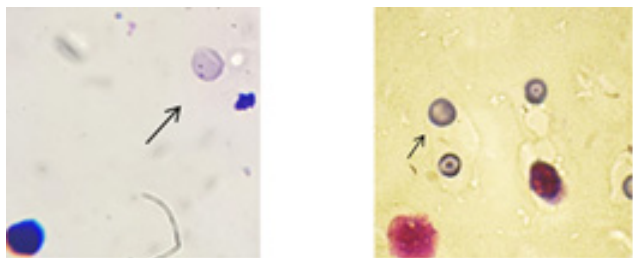

Figure (1): A : a normal polychromatic Erythrocyte (PCE) from the bone marrow of a rat of the negative control group (May-Grunewald + Giemsa) 100x

B: polychromatic Erythrocyte (PCE) containing a micronuclei from the bone marrow of a rat of the group treated with ciprofloxacin drug (CFX) (May- Grunwald + Giemsa) X100

Number of Micronuclei Mean % ± S.E	**Number of polychromatic erythrocytes with Micronuclei Mean % ± S.E**	**Percentage of polychromatic erythrocytes PCE_s%))**	**No of animals**	**Treatment Dosage Mg.kg^{-1} body weight**
6.800 ±0.860	4.400±0.400	9.84	5	Negative control
124.40 ±5.22**	81.20± 2.92 **	18.93	5	Mitomycin-c 1
21.40±1.50**	14.80±1.07**	11.13	5	Ciprofloxacin 93.5
**4.91±49.60	32.60±2.91**	11.22	5	Ciprofloxacin 187

Table (1) shows the micronuclei in the bone marrow of white male rats after treatment with different doses of CFX, negative control and positive control (MMC)

*** Significance at the probability level P≤ 0.05 (t-test)**

**** Significance at the probability level P≤ 0.01 (t-test)**

This test was used in the current study to assess the genotoxicity and cytotoxicity of ciprofloxacin drug (CFX). The table shows an increase in the percentage of polychromatic erythrocytes (PCES) in bone marrow of white rats at treatment with dose 93.5 mg.kg^{-1} of body weight by 11.13%. Treatment with a dose of 187 mg.kg^{-1} of body weight gave a slight increase by 11.22%, and these are results which show a little change from the negative control of 9.84. This indicates that the abovementioned drug may have a cytotoxicity. It also indicates to the effect of the mentioned two doses of the drug in stimulating the cells generating erythrocytes to increse division in order to produce more cells and replace lost cells as a result of the toxic effect of the drug in the treated animal. As for the positive control , it was the largest percentage compared to the negative control and treatment with a percentage at 18.93%. The results of the table showed that the arithmetic mean of micronuclei with the dose 187 mg.kg^{-1} of body weight was (4.91 ± 49.60) which is of significance compared to negative control (0.860 ± 6.800) and the arithmetic mean with the dose 93.5 mg.kg^{-1} of body weight was (21.40 ± 1.50) which is of significance compared to negative control 0.860 ± 6.800. This indicates that the drug may be genotoxic because it caused an increase in the frequency of micronuclei, and this increase is related to the increase in the concentration of the drug.

The results of the table showed that the arithmetic mean of polychromatic erythrocytes containing micronuclei when treated with the dose 187 mg.kg^{-1} of body weight was of significance at (2.91 ± 32.60) while the treatment with the dose 93.5 mg.kg^{-1} of body weight was of less significance with (14.80 ± 1.07) compared to negative control of (4.400 ± 0.400) This leads to the assumption of genotoxicity of the drug by its ability to develop micronuclei in polychromatic erythrocytes. These results are consistent with studies conducted to assess and measure the genotoxicity of the drug [7-9]. It is possible that the mechanism for the formation of micronuclei by ciprofloxacin drug in the bone marrow of treated rats is that the drug inhibits Topoisomerase II enzyme in the treated rats. Cyprofloxacin is known to be anti-bacterial by inhibiting DNA gyrase. And this enzyme is not present in eukaryotic cells but may be functionally and precipitatively connected toTopoisomerase II enzyme for eukaryotic cells [10]. The Topoisomerase II and DNA gyrase are essential for life, as they play a role in the formation of active chromatin synthesis, limiting strand twisting and dismantles of interlocking strands during DNA replication, and contributing to chromosome depletion during mitosis and chromosomal separation during the anaphase [11]. Inhibition of the Topoisomerase

II enzyme prolongs the metaphase and interferes with the dissociation of sister chromatids in the anaphase, but does not prevent the cell from that causing chromosomal abnormalities and indivisibility, thus leading to the formation of fine micronuclei [12]. The micronucleus test is widely used in many exploratory research to understand the potential underlying mechanism of genotoxicity. Its simplicity and applicability to a wide range of cell types inside and outside the body makes it a flexible tool for understanding the keys to genotoxicity and its effect on humans. The micronucleus test has shown to be one of the preferred methods for estimating chromosomal damage since it has been able to measure both total chromosomal loss or chromosomal fractions in a reliable manner because only micronuclei can be expressed in cells that have completed nuclear division. There is a hypothesis stating that micronuclei can have predictive value for cancer, as well as genetic mutations, and therefore replace chromosomal aberrations as a life indicator of cancer risk [13].

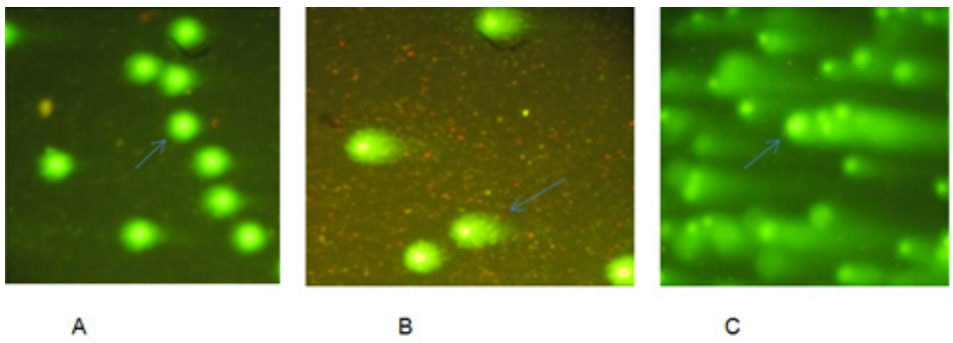

Figure (2) A: Estimating the induction of DNA damage in bone marrow, spleen and liver cells of male white rats using the Comet assay estimation technique . B: shows the natural DNA of a cell from the negative control group (S.G) 10X C : shows abnormal DNA from the treatment group (CFX) (S.G) 10x

The figures show different levels of DNA damage in cells of both the negative control group and the Ciprofloxacin treatment group. The table clarifies the percentages of cells with damage, mean values , standard error in the bone marrow DNA of the white rats treated with the drug, and negative control and positive control (MMC)

Table (2): Percentage of cells with damage and levels of damage in the DNA of bone marrow of white rats treated with the drug, and negative control and positive control (MMC)

Cells with damaged DNA Mean % ± S.E	Percentage of cells with damage	Total number of cells examined	No. of Animals	Treatment Dosage $Mg.kg^{-1}$ body weight
0.510 ± 2.400	2.4	500	5	Negative control
3.39± 31.40**	31.4	500	5	Mitomycin-c 1
2.03± 13.20 **	13.4	500	5	Ciprofloxacin 93.5
19.80± 2.92 **	19.8	500	5	Ciprofloxacin 187

*** Significance at the probability level P≤ 0.05 (t-test)**

**** Significance at the probability level P≤ 0.01 (t-test)**

Table (3): Percentage of cells with damage, levels of damage in the spleen DNA of white rats treated with the drug, and negative control and positive control (MMC).

Cells with damaged DNA Mean % ± S.E	Percentage of cells with damage	Total number of cells examined	No. of Animals	Treatment Dosage $Mg.kg^{-1}$ body weight
0.510 ± 2.400	2.4	500	5	Negative control
2.08± 33.20**	33.2	500	5	Mitomycin-c 1
2.51± 14.00 **	14	500	5	Ciprofloxacin 93.5
21.20± 2.58 **	21.2	500	5	Ciprofloxacin 187

*** Significance at the probability level P≤ 0.05 (t-test)**

**** Significance at the probability level P≤ 0.01 (t-test)**

Table (4): Percentage of cells with damage , levels of damage in the liver DNA of white rats treated with the drug, and negative control and positive control (MMC).

Cells with damaged DNA Mean % ± S.E	Percentage of cells with damage	Total number of cells examined	No. of Animals	Treatment Dosage $Mg.kg^{-1}$ body weight
0.510 ± 3.600	3.6	500	5	Negative control
6.23± 35.80**	35.8	500	5	Mitomycin-c 1
16.20± 1.02 **	16.2	500	5	Ciprofloxacin 93.5
2.54± 23.4 **	23.4	500	5	Ciprofloxacin 187

*** Significance at the probability level P≤ 0.05 (t-test)**

**** Significance at the probability level P≤ 0.01 (t-test)**

Single Cell Gel Electrophoresis tecnique or Comet Assay technique is a direct method for identifying DNA damage in interphase cells. The tail represents the amount of damage done in DNA. This technique is particularly sensitive in the recognition of individual sequence fractures, and the locations of repair by cutting in single cells compared to conventional ways of identifying DNA damage. It is useful to show DNA damage in a single cell. It is a direct, sensitive, simple, fast, and effective technique used in studies of genotoxicology. It has been generally assumed that SCGE technique, which is conducted under alkaline conditions, recognizes the individual sequence fractions and the location of the alkalines in DNA [14]. The ability of DNA to migrate depends on the size and number of fractures in the molecule as a result of a particular factor [15].

The comet estimation technique requires high-level viability cells approximating 95%, and low levels of DNA damage were found in the negative control group during the experiment.

The results of Table (2) show the percentage of cells with comet tails that represent the affected DNA cells for both negative and positive control and treatment

groups with dose 93.5 and 187 mg.kg^{-1} body weight of ciprofloxacin in bone marrow cells. The results showed an increase in percentages of damaged DNA cells and the two groups treated with 187, 93.5 mg.kg^{-1} body weight respectively.

The results of the table also show mean values of DNA damage in bone marrow cells of white male rats. The results clarified that the treatment with the dose 187 mg.kg^{-1} of body weight was significant with (19.80 ± 2.92) compared with the negative control (0.510 ± 2.400) , while the treatment with the dose 93.5 mg.kg^{-1} of body weight was significantly less than (2.03 ± 13.20) compared to the negative control of (0.510 ± 2.400).

The results of Table (3) show the percentage of cells with comet tails that represent the affected DNA cells of both negative and positive control and that treated with doses 93.5 and 187 mg.kg^{-1} of body weight with ciprofloxacin drug in spleen cells. The results show an increase in the percentage of damaged DNA cells for the positive control group (treated with MMC drug) and the two groups treated with the drug 187, 93.5 mg.kg^{-1} of body weight respectively.

The results of Table (4) show the mean values of DNA damage in the spleen cells of white male rats. The results clarify that the treatment with dose of 187 mg.kg^{-1} of body weight was significant with (21.20 ± 2.58) compared to negative control with (0.66 ± 3.20), while the dose treatment of 93.5 mg.kg^{-1} of body weight was less significant and reached 2.51 ± 14.00 compared to negative control with (0.510 ± 2.400)

The results of the table also show the damage caused in the DNA liver cells in white rats. The percentage of DNA cells with damage was higher than in bone marrow and spleen blood in both negative and positive control groups and treatment group with doses 93.5 and 187 mg.kg^{-1} body weight with ciprofloxacin. The mean values are significant in the treatment dose of 187 mg.kg^{-1} body weight. It was (2.54 ± 23.4) compared to negative control group of (0.510 ± 3.600) while the dose of 93.5 mg.kg^{-1} body weight was significantly less than (1.02 ± 16.20) compared to the negative control group with (0.510 ± 3.600). These values are higher than those of DNA in bone marrow and spleen. This means that both the percentage of cells with comet tails and damage to the liver cell DNA are higher than in bone marrow and spleen cells. This may be due to the fact that the detoxification device is present in the liver which is a chromosomal device and is part of the defense system of the body. Most of the material is metabolized inside the liver, therefore, it converts it by introducing chemical changes so that it can be destroyed and get rid of its poisonous effect and highlighted in several operations as a useless metabolic material. This drug is partially metabolized in the liver by modifying the pepirazineel group into at least four metabolites. These metabolites, identified as diethyline ciprofloxacin, sulfo-ciprofloxacin, oxo-ciprofloxacin and cyprofloxacin N-acetyl, show a weaker microbiological activity than the initial form of the drug, but a similar or stronger activity for some other quinolones [16].

The results of the tables for MMC treatment were significant for liver cell DNA, and bone marrow and spleen cells DNA. Rjiba *et al* have shown in their study that the effect of MMC drug was high [17]. The DNA damage caused by CFX drug may be due to its ability to release free radicals (void of oxygen) [18]. The oxygen free radicals attack the DNA causing mutations [19].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

[1] McKellar QA, Gibson I, Monteiro A, Bregante M. Pharmacokinetics of enrofloxacin and danofloxacin in plasma inflammatory exudate and bronchial secretions of calves following subcutaneous administration. Antimicrobial Agents And Chemotherapy. 1999;43:1988–1992.

[2] Davis H, McGoodwin, Reed ETG. Anaphylactoid reactions reported after treatment with ciprofloxacin. Ann Intern Med. 1989;**15**(12):1041–1043.

[3] Hussy P, Maass G, Tummler B. Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase alpha primase complex topoisomerases I and II and growth of mammalian lymphoblasts. Antimicrobial Agents and Chemotherapy. 1986;29(6):1073-1078.

[4] Vancutsem PM, Babish JG, Schwark WS.

The fluoroquinolone antimicrobials: structure, antimicrobial acitivity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet. 1990;80:173-186.

[5] Schmid W. The micronucleus test. Mutat. Res. 1975;31:9-15.

[6] Dhawan AN, Mathur PK. The effect of smoking and eating habits on DNA damage in Indian population as measured in the Comet assay. Mutation Res. 2001;474(1-2):121-8.

[7] Olajyigbe O, Alabi O, Anaba U. Induction of micronucleus and abnormal sperm cells in albino mice exposed to ciprofloxacin. Der Pharmacia Lettre**.** 2011;3(2):438-445.

[8] Khadra A, Pinelli E, Lacroix MZ, Bousquet-Melou A, Hamdi H, Merlina G, Guiresse M, Hafidi M. Assessment of The genotoxicity of quinolone and fluoroquinolones contaminated soil with the Vicia faba micronucleus test. Ecotoxicology and Enviromental Safety. 2011;76**:**187-192.

[9] Käufer MG, Haddad T, Bergheim M, Gminski R, Gupta P, Mathur N, Kümmerer K, Mersch-Sundermann V. Genotoxic effect of ciprofloxacin during photolytic decomposition monitored by the in vitro micronucleus test (MNvit) in HepG2 cells. Environmental Science and Pollution Research. 2012;19:1719–1727.

[10] Wyckoff E, Natalie DM, Nolan JM, Lee M, Hsieh TS. Structure of the *Drosophila* DNA topoisomerase-II gene: nucleotide sequence and homology among topoisomerase-II, J. Mol. Biol. 1989;205.

[11] Suh B, Lorber B. Quinolones, MEDICAL CLINICS OF NORTH AMERICA. 1995;79

[12] Downes CS, Mullinger AM, Johnson RT. Inhibitors of DNA topoisomerase II prevent chromatid separation in mammalian cells but do not prevent exit from mitosis. Proc.Natl.Acad. Sci.1991;88:8895-8899.

[13] Ardema MJ, Snyder RD, Spicer C. SFTG international collaborative study on in vitro micronucleus test III Using CHO cells. Mutat. Res. 2006;607:61–87.

[14] Singh, N.P.; McCoy, M.T. and Tice, R.R. (1988). Schneider ELA. Simple technique for quantification of low levels of DNA damages in individual cells. *ExpCell Res* **175**:184–91.

[15] Garaj-Vrhovac V, Zeljezic D. Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides. Toxicology. 2001;165:153-162.

[16] Al-Omar AM. Ciprofloxacin: Drug Metabolism and Pharmacokinetic Profile. Profiles of Drug Substances, Excipients and Related Methodology. 2005;31:209 - 214.

[20] Rjiba TK, Boussema AI, Guedri Y, Achour A, Bacha H, Abid S. Role of recombinant human erythropoietin in Mitomycin C-induced genotoxicity analysis of DNA fragmentation chromosome aberrations and micronuclei in rat bone-marrow cells. Mutation Research. 2013;753:48-53.

[18] Gurbay A, Gonthier B, Signorini N. Ciprofloxacin-induce DNA damage in primary culture of rat astrocytes and protection by Vitamin E. Neurotoxicology. 2006;27(1):6-10.

[19] Arriaga-Alba M, Sánchez RR, Pérez NGR, Navarrete JS, Paz RF, Montoya-Estrada A, Gomez JJH. Comparative study of the antimutagenic properties of vitamin C and E on mutations induced by norfloxacin. BMC Pharmacol. 2008;8(1):2.