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Induction of micronucleus and abnormal sperm cells in albino mice exposed to ciprofloxacin

Olufunmiso OLAJUYIGBE[1, 2], Okunola ALABI*[2] and Udochisom ANABA[2]

[1]*Phytomedicine Research Centre, University of Fort Hare, Alice, 5700, South Africa*
[2]*Department of Biosciences and Biotechnology, Babcock University, PMB 21244, Ikeja, Lagos, Nigeria*

ABSTRACT

The mammalian in vivo micronucleus and sperm morphology assays are widely used as part of the battery of assays for genotoxicity testing required during the development of new drugs. Genotoxicity data on Ciprofloxacin in mammalian system are very limited and mostly contradictory. This study provides more data by assessing the genotoxic potential of ciprofloxacin using mouse micronucleus and sperm morphology assays. 320µg/ml, 160µg/ml, 80µg/ml, 32µg/ml and 16µg/ml of ciprofloxacin were daily administered intraperitoneally for 5 days, after which the animals were sacrificed for the assays. The results showed a statistically significant ($p<0.05$) concentration-dependent induction of micronucleus and abnormal sperm morphology in the exposed mice. Result indicates a warning signal to injudicious and indiscriminate use of the drug

Keywords: Ciprofloxacin, genotoxicity, micronucleus, sperm morphology, mutagenic.

INTRODUCTION

Ciprofloxacin (CFX) is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class.[1][2] It is a second generation fluoroquinolone with a broader antibacterial spectrum of activity than other fluoroquinolones.[3] The CFX is among the most commonly used antibiotics for different kind of infections caused by susceptible bacteria in lower respiratory tract, skin, bone, joint, urinary tract and infectious diarrhea.[4] The fluoroquinolones exhibit concentration-dependent bactericidal activity and exert their activity by binding to bacterial topoisomerases II (DNA gyrase) and IV. By binding to these bacterial target sites, quinolones

interfere with DNA replication, repair, and transcription as well as with other cellular functions, rapidly leading to bacteria death.[5] Therefore, these drugs are widely used in clinical practice. CFX is contraindicated in pregnancy and children[6] because it has many adverse effects such as hepatotoxicity,[7] nephrotoxicity,[8] androtoxicity, reproductive developmental toxicity, carcinogenicity and phototoxicity.[9][10]

Genotoxic potential of drugs is assessed as part of the safety evaluation process and genotoxicity assays have become an integral component of drug regulatory requirements.[11] The induction of genetic damage is a critical step in the development of different genetic diseases in future generations, birth defects and contributes to somatic diseases such as cancer in the present generation. Therefore it is necessary to assess the genotoxic potential of drugs to evaluate their ability to cause cancer and other defects. Limited data however exist in literature about the genotoxicity of CFX in mammalian system. *In vitro* genotoxicity of CFX has been demonstrated with sister chromatid exchange (SCE) and unscheduled DNA synthesis,[12] while *in vivo* genotoxicity of CFX has been demonstrated with the micronucleus test[13] and chromosomal aberrations in lymphocytes of humans,[14] mice [15] and rats.[16] However, genotoxicity data on CFX in mammalian test system are contradictory.

As a result of very frequent usage of CFX nowadays which is partly due to on-the-counter purchases, while quacks and doctors are liberally using the drug unchecked,[17][3] there is a need for more genotoxic data to effectively conclude on the genotoxicity status of CFX. Hence, this study was designed to investigate the effects of CFX on micronucleus (MN) and sperm morphology in albino mice.

MATERIALS AND METHODS

Test substance

CFX used was of pharmaceutical grade purchased from Fidson Pharmaceutical Company, Lagos, Nigeria. Five concentrations of 320µg/ml, 160µg/ml, 80µg/ml, 32µg/ml and 16µg/ml were utilized in this study. 320µg/ml of CFX has been determined to cause significant cell damage compared to control in our pilot study. Cyclophosphamide (20mg/kgbw) and distilled water were used as positive and negative controls respectively.

Biological materials

Young male Swiss-albino mice of 6-10weeks old were obtained from Nigeria Institute of Medical Research, Lagos, Nigeria. They were acclimatized for two weeks in a pathogen-free, well ventilated room at the Department of Biosciences and Biotechnology, Babcock University, Ogun State, Nigeria. They were supplied with uninterrupted water and food and maintained in the same room throughout the period of the study. Animals 8weeks old were used for MN assay while mice of 12-14weeks were used for sperm morphology assay.

Micronucleus (MN) assay

Five mice per concentration were daily injected intraperitoneally (IP) with a single dose of

0.5ml of each concentration of CFX for 5 consecutive days. Negative (distilled water) and positive (cyclophosphamide, 20mg/kg body weight) controls were also administered. MN was carried out as previously described by Bakare et al.[18] Briefly, the femurs were surgically removed after the animals had been sacrificed by cervical dislocation. The bone marrow was flushed from the femurs with Foetal Bovine serum (Sigma Aldrich Cheme GmbH, Germany). The cells were centrifuged for five minutes at 2000rpm and the slides were subsequently stained with May-Grunwald and Giemsa stains respectively. About 1000cells/animal were scored for micronuclei in polychromatic erythrocytes (MNPCE) and normochromatic erythrocytes (MNNCE). The PCE: NCE ratio in every 1000cells counted was analyzed.

Sperm morphology assay

The method of Wyrobek et al.[19] was utilized. The same number of animals, test sample concentrations and exposure route and duration of administration as MN were utilized in this assay. 35days exposure period was considered because spermatogenesis takes about 34.5 days to complete in mice. Sperms were sampled from the caudal epididymes after the animals had been sacrificed by cervical dislocation. Two sperm suspensions were prepared from the caudal of each testis by mincing the caudal in physiological saline. The prepared slides were stained with 1% Eosine Y for 45mins after which the slides were air dried. 800 sperm cells/mouse were assessed for morphological abnormalities under oil immersion at 1000x according to the criteria of Wyrobek and Bruce [20].

Statistical analysis

The SPSS® 14.0 statistical package was used for data analysis. Significance at the different dose-level of each assay was tested by using the Dunett t- test. ANOVA was used for testing significance. Differences between the negative control-group and individual dose-groups were analyzed at the 0.05 and 0.001 probability levels.

RESULTS

Micronucleus assay

Figure 1 shows the MN induced in the bone marrow cells after exposure of mice to the different concentrations of the test sample. The induction of MN in the test concentrations as compared with the negative control showed a statistically significant ($p<0.05$), concentration-dependent increase in MN at all concentrations (Table 1). The size and position of micronucleus in the cytoplasm showed slight variation and between 1-3 MN were observed in the exposed cells. The maximum induction of MN was at the highest concentration of 320µg/ml. There was statistically significant ($p<0.05$) increase in NCE at all concentrations tested (Figure 2).

Sperm morphology assay

Various abnormalities in sperm morphology were observed. The frequency of abnormal sperm cells in the negative control was 2.00%. There were 8.5%, 5.5%, 5.3%, 5.1% and 3.95% of abnormal sperm cells at 320µg/ml, 160µg/ml, 80µg/ml, 32µg/ml and 16µg/ml tested

concentrations respectively. In the treated animals, the frequency of abnormal sperm cells was statistically significant ($p<0.05$) and concentration-dependent at all concentrations. The statistical analysis confirmed that the inter-animal variations were insignificant but the inter-concentration variations were significant ($p<0.05$). Generally, folded sperm cells were more frequent than the remaining types of aberration in all experimental groups (Figure 3). Table 2 shows the effect of the different concentrations of Ciprofloxacin on the sperm cells.

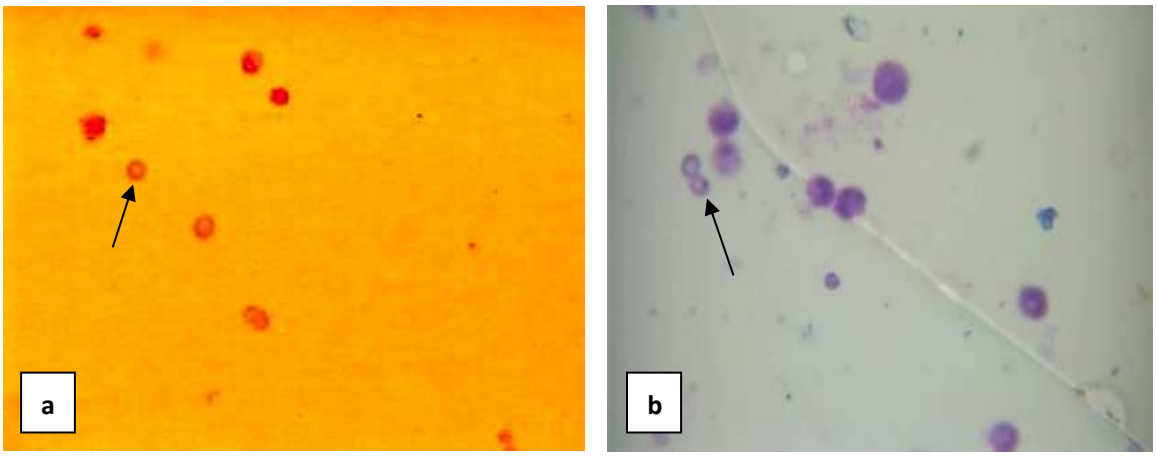

Figure1: Micronuclei induced in mice exposed to different concentrations of Ciprofloxacin (a) Normal PCE (b) MNPCE. Magnification x1000.

Table 1: MN induction in mouse bone marrow cells intraperitoneally exposed to different concentrations of Ciprofloxacin

Test sample	Concentration	Number of cells scored	No of MNPCEs	Mean value ± S.D
Negative control (distilled water)	0%	1000	26	6.25 ±0.96
Ciprofloxacin	16µg/ml	1000	39	9.75 ±0.96*
	32µg/ml	1000	46	11.50 ±1.29*
	80µg/ml	1000	79	19.75 ±1.50*
	160µg/ml	1000	98	24.50 ±0.82*
	320µg/ml	1000	106	26.1 ±1.26*
Positive control (cyclophosphamide)	20mg/kg bwt	1000	141	28.31 ±0.60*

**Significant at p< 0.001.*

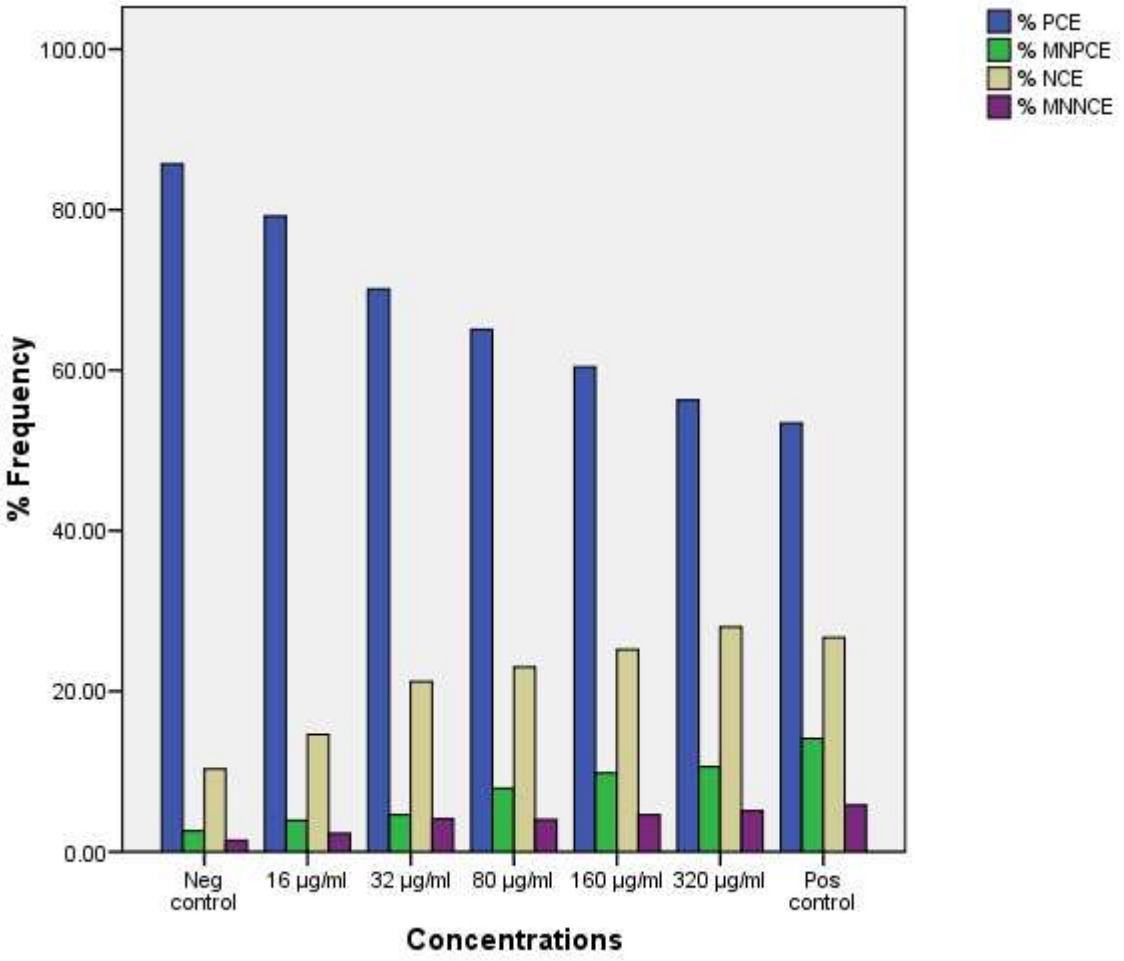

Figure 2: The percentages of PCE, MNPCE, NCE and MNNCE induced by different concentrations of Ciprofloxacin.

Table 2: Summary of abnormal sperm cells induced by different concentrations of Ciprofloxacin in mice after 5 weeks exposure

Concentrations (%)	Number of Animals used	Number of Sperms counted	Number of abnormal sperms	% Frequency of abnormality
Distilled water	5	4000	110	2.75
16µg/ml	5	4000	232	5.80
32µg/ml	5	4000	318	7.95*
80µg/ml	5	4000	750	18.75*
160µg/ml	5	4000	1122	28.05*
320µg/ml	5	4000	1200	30.00*
Cyclophosphamide (20mg/kgbwt)	5	4000	1135	28.38*

**Significant at $p < 0.05$.*

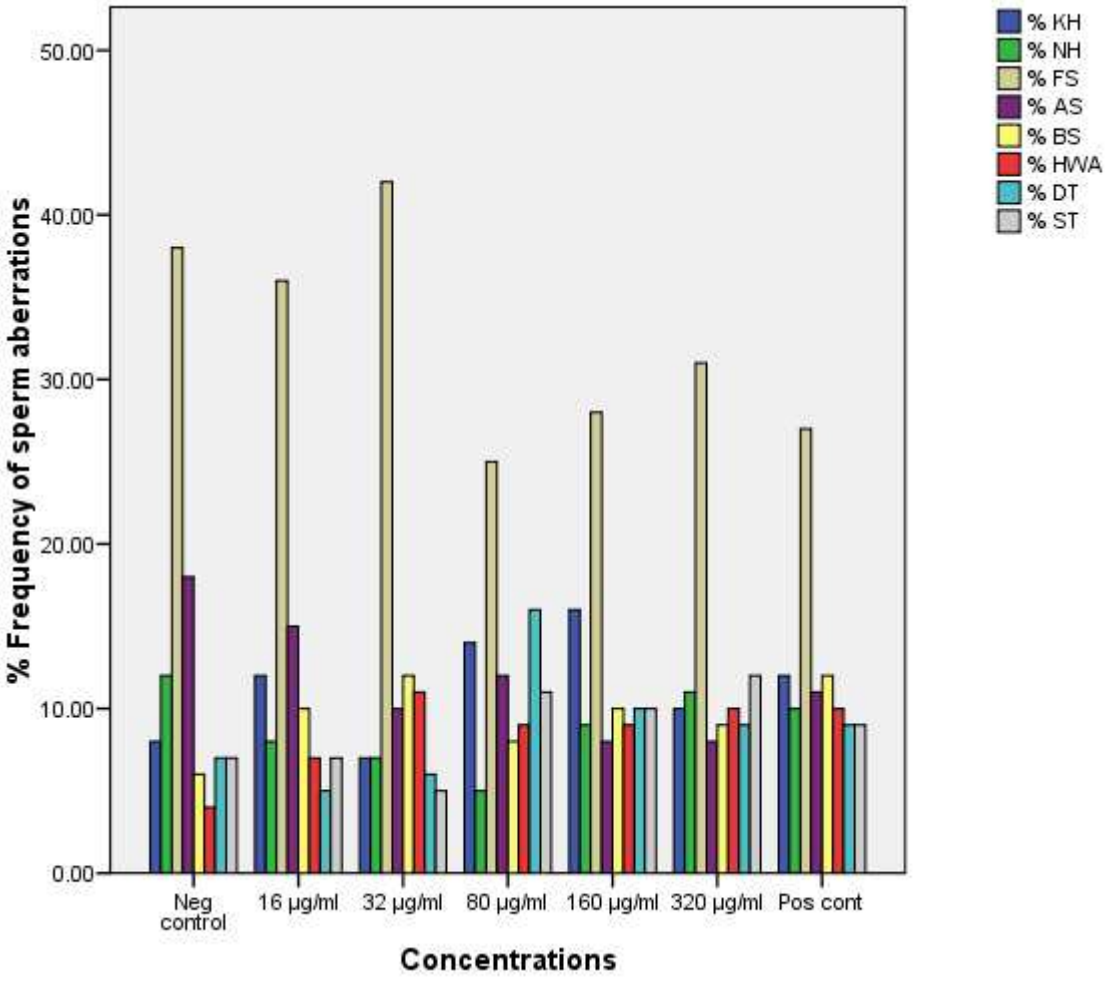

Figure 3: Percentages of each type of sperm aberration induced by different concentrations of Ciprofloxacin.

KH- Knubbed hook, NH- No hook, FS- Folded sperm, AS- Amorphous-head sperm, BS- Banana shape sperm, HWA- Hook at wrong angle, DT- Double tail, ST- Short tail.

DISCUSSION

The *in vivo* micronucleus test in bone marrow[21] or peripheral blood erythrocytes[22] and sperm morphology assay are widely used as short-term assays for the detection of agents able to induce chromosome aberrations in somatic cells and alter spermatogenesis respectively.[23-25] The tests have also been shown to have good predictive potential for the identification of germ cell mutagens.[20][26] In this study, the induction of micronuclei and abnormal sperm morphology by ciprofloxacin was carried out to test its genotoxicity and mutagenicity.

The result of MN assay in this study showed that CFX is capable of inducing aneugenic and clastogenic effects in exposed mice. The micronucleus assay is devised primarily for evaluating the ability of test agents to induce structural and/or numerical chromosomal damage. Both types of damages are associated with the appearance and/or progression of

tumors, and with adverse reproductive and developmental outcome.[27] A possible mechanism for the induction of micronuclei in the mouse bone marrow cells is that CFX inhibited topoisomerase II in the exposed mice. CFX has been known to exert its antibacterial effect by inhibiting DNA gyrase in bacteria. Although gyrase is not found in eukaryotic cells, however, gyrase is functionally and structurally related to the eukaryotic topoisomerase II.[28] DNA gyrase or topoisomerase II, which is essential to life, plays a role in establishing the structure of transcriptionally active chromatin, reducing torsional strain and resolving intertwined strands during DNA replication, condensation of chromosomes during mitosis, and chromosome disjunction at anaphase.[5] Inhibition of topoisomerase II prolongs metaphase and interferes with the separation of sister chromatids at anaphase, but does not prevent cells from undergoing a cleavage that results in chromosome abnormalities and non-disjunction,[29] thereby can lead to the formation of micronucleus. This shows that the tested compound is capable of chemical induction of DNA damage in the exposed animals.

The criteria for positive response were satisfied in the sperm morphology assay with a significant increase in abnormal sperm morphology at all treated concentrations compared with the negative control. Evidences from previous reports in sperm morphology show that, alteration in sperm morphology is as a result of alterations in sperm chromatin compaction which is possibly due to a protamine deficiency or incomplete protamine sulfhydryl oxidation.[30][31][32] It has also been shown that increase in abnormal sperm cells in male germ cells exposed in vivo to a test sample demonstrates the sample ability to alter spermatogenesis.[19] Formation of abnormal sperm cell is suggested to be as a result of mistakes in the packaging of the genetic material and an agent that induces abnormality is an agent that interferes with the integrity of the DNA itself or with the expression of the genetic material.[20]

These results are in accordance with previous reports.[12-16] The results of this study, together with other published data, indicate that the antimicrobial drug, CFX, is able to induce both mutagenic and genotoxic effects in animal models. Result indicates a warning signal to injudicious and indiscriminate use of the drug. The observed genotoxicity can possibly occur in humans who abuse the dosage or are subjected to a long term usage of CFX.

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