Anandpriya. *et al.* / *International Journal of Pharmacy & Therapeutics*, 4(4), 2013, 265-269.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EVALUATION OF GENETIC AND MOLECULAR TOXIC EFFECTS OF CIPROFLOXACIN

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ABSTRACT

Ciprofloxacin is a Fluoroquinolone antibiotic, widely used for the treatment of various bacterial infections. Inspite of its therapeutic utility, it has several side-effects, like developmental toxicity in growing children. As there is a lacuna in the genetic and cytotoxic studies, we have planned to do this study. In this study we have evaluated the Cytotoxic and Genotoxic effect of Ciprofloxacin (CFX), on cultured human lymphocytes by an *invitro* method. This drug was found to have Cytotoxic and Genotoxic effects on human lymphocyte cultures *in vitro.* This might be one of the reasons for various ill effects of Ciprofloxacin.

Key Words:- Ciprofloxacin, Cytotoxicity, Genotoxicity.

INTRODUCTION

Ciprofloxacin is a Fluorinated 4-quinolone antibiotic, have broad anti- microbial activity widely used for the treatment of various bacterial infections, especially by gram negative bacterias and some gram positive bacterias (Reeves DS *et al* 1994) . Inspite of its therapeutic utility, it has several side effects. Previous studies have reported that Ciprofloxacin has toxic effects on Musculoskeletal system, Central Nervous system, Cardiovascular system, Gastrointestinal system, can cause Reproductive and developmental toxicity (Stahlmann R *et al* 1998, Schluter G *et al* 1987, Weyers AI *et al* 2002, Yong-Taek J *et al* 2003, Yoko Kashida *et al*, Minta M *et al* 2005, Stahlmann R *et al* 2002). Mechanism of action of Ciprofloxacin is, it is a potent bactericidal drug. They target bacterial DNA gyrase (Dalhoff A *et al* 2003, Suh B *et al* 1995)

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and Topoisomerase IV enzyme resulting in bacterial cell death (Barrett JF *et al* 1990). DNAgyrase is mainly inhibited in Gram negative bacterias. Topoisomerase IV is mainly inhibited in Gram positive bacterias. Topoisomerase IV-separate the interlinked daughter DNA molecule which are products of DNA replication. Double stranded DNA should undergo separation, so that individual strand can undergo replication. While separation, excessive super coiling occurs in front of point of separation. Bacterial DNAgyrase relieves the supercoiling by nicking and resealing action. It has 2 subunits A and B, encoded by gyr A and gyr B gene. A subunit takes care of nicking action. Ciprofloxacin acts at this site. Mutation of gene encoding for A subunit is responsible for resistance against ciprofloxacin. Ciprofloxacin by inhibiting bacterial DNA gyrase, prevent bacterial DNA replication during growth and reproduction. In addition, inhibition of DNA gyrase leads to extensive filamentation, vacuole formation and degradation of chromosomal DNA – all these result in bacterial cell death. Thus Ciprofloxacin interferes with DNA synthesis, DNA replication, repair, transcription and

other cellular function thus resulting in bacterial cell death. Eukaryotic cells do not contain DNA gyrase but contain conceptually similar DNA Topoisomerase ll enzyme (Barrett JF *et al* 1990, Smith JT *et al* 1984, Gorla N *et al* 1999). Previous studies state that only very high concentration of Ciprofloxacin is required to inhibit this mammalian Topoisomerase ll (Anupama M *et al* 2010, Hussy P *et al* 1986, Forsgren A *et al* 1987). There are few other studies which state that Ciprofloxacin can inhibit these enzymes even at lower concentration (Ikbal M *et al* 2004, Ambulkar PS *et al* 2009, Gorla *N et al 1999*, Mukherjee A *et al* 1993). In our study we have used lower concentration of Ciprofloxacin to find out whether it has Cytotoxic and Genotoxic effect on normal human lymphocytes due to cross reactivity by acting on this mammalian Topoisomerase ll. The present study has been done to evaluate the Cytotoxic and Genotoxic effect of Ciprofloxacin on cultured human lymphocyte, using 3 different concentrations of Ciprofloxacin and compared it with that of control. Since Ciprofloxacin is widely used in India, and as there is lacunae in the genetic and Cytotoxic studies, we planned to do this study.

AIM AND OBJECTIVE

To assess the Cytotoxic and Genotoxic Effect of Ciprofloxacin and compare it with that of the control, on cultured human lymphocytes by an *invitro* method.

MATERIALS AND METHODS

After obtaining Ethical committee approval, this study was done by an *invitro* method with conventional cytogenetic techniques using parameters like Mitotic Index (MI), Replicative Index (RI), and Sister Chromatid Exchange (SCE) as end points. 5ml of peripheral venous blood sample was collected from a healthy female volunteer of age 35yrs, who was not pregnant, a non smoker, with no metabolic and infectious diseases, and no history of recent or long term drug intake. 4 lymphocyte cultures were set up by adding 0.5ml of heparinized whole blood with 8ml of RPMI 1640 medium (*Roswell Park Memorial Institute medium)* (80%), 2ml of Fetal Bovine Serum (20%), 250µl Phytohemagglutinin and 5-Bromo 2Y-deoxyuridine (BrdU)- 100µl (Ambulkar PS *et al* 2009,Khan AA *et al* 1998). One culture was kept as a control (A). To the other three experimental cultures (B), (C) and (D), Ciprofloxacin 5 µg/ml, 25 µg/ml, and 50 µg/ml were added respectively. All 4 Cultures were incubated at 37^0C for 72 hrs in complete darkness. 3hrs prior to harvesting Colchicine 100µg was added to all the 4 cultures to arrest the cells at Metaphase. After 1½ hrs the cultures harvested were transferred to centrifuge tubes and treated with hypotonic solution, Potassium Chloride solution. After 20mins, the tubes were centrifuged for 1000 rpm/10mins. Supernatant was discarded leaving the buffy coat which contain the cells. Then Carnoy's fixative (3:1 mixture of methanol / acetic acid) was added to the cells. Slides were prepared with the cell suspension. For Staining, MI slides were stained with Giemsa stain. For SCE and RI, slides were stained initially with Fluorescence dye (Hoecht dye) in dark and then with Giemsa stain. Stained slides were dried and observed under light microscope.

Determination of MI: To determine MI, under light microscope 1000 cells were scored randomly from each slide and metaphases were identified.
Mitotic Index was calculated with the formula

$$MI = \frac{\text{Number of Metaphases}}{\text{Number of Interphases}} \times 100$$

Determination of SCE: To determine SCE, under light microscope a total of 50 good metaphases from each slide were scored and number of SCE per metaphase was noted under 100x.

Determination of RI: To determine RI, 50 metaphases were randomly scored from each slide.
RI was calculated using the formula:

$$RI = \frac{M1 + 2\,M2 + 3\,M3}{N}$$

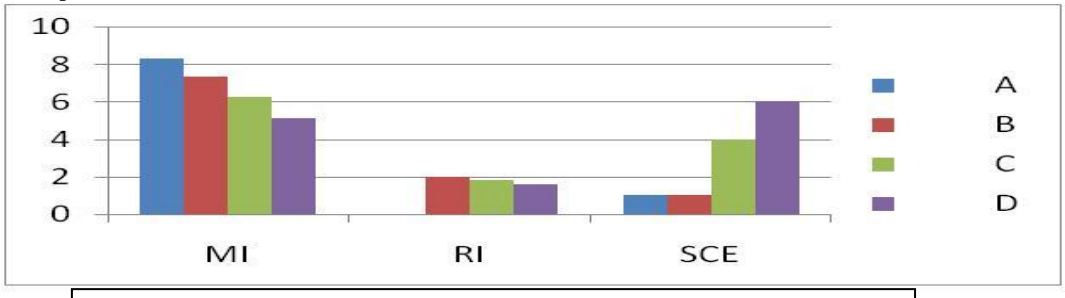
M1, M2, and M3 are metaphases corresponding to the first, second and third cycles respectively.
N – Total number of Metaphases scored.

RESULTS

Table 1. Comparison of Cytogenetic Analysis of Peripheral Blood Lymphocyte between the Control and Experimental Group

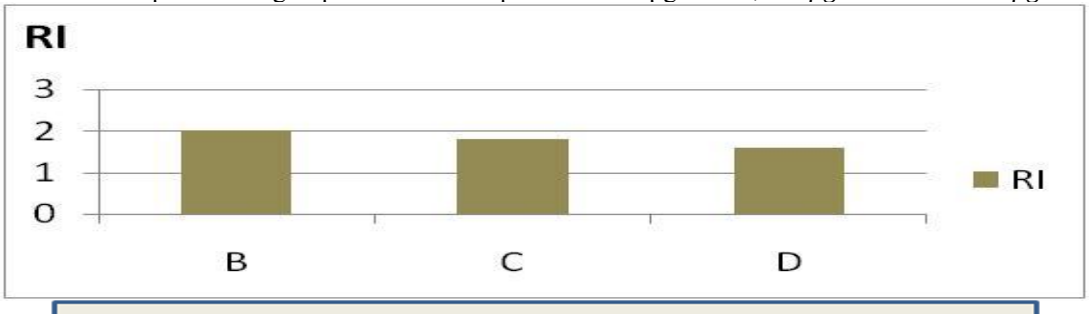
GROUP	MI	RI	SCE
CONTROL -A	8.29 %	-----	0 to 1
EXPERIMENTAL GROUP			
B-5µg/ml	7.33 %	2	0 to 1
C-25µg/ml	6.27 %	1.8	0 to 4
D-50µg/ml	5.13 %	1.6	0 to 6

Bar Diagram showing Comparison of Cytogenetic Analysis of Peripheral Blood Lymphocyte between the Control and Experimental Group



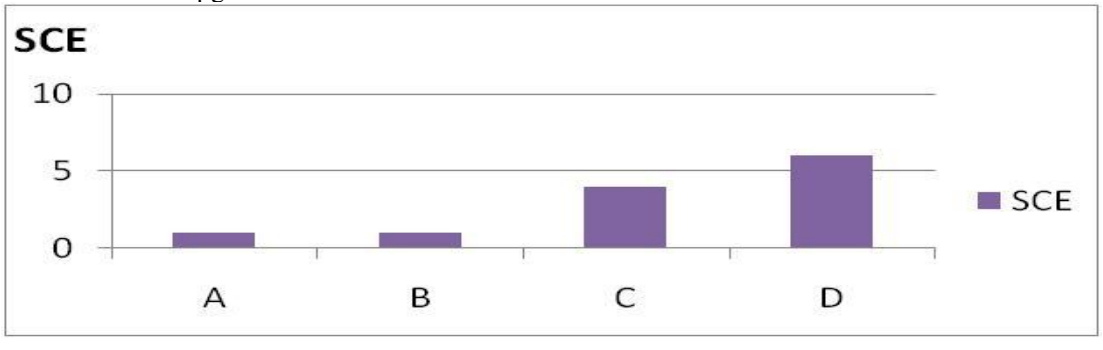
A – Control, B - 5µg/ml, C - 25µg/ml, D - 50µg/ml

Replicative index in the experimental group treated with Ciprofloxacin 5 µg/ml – 2, 25µg/ml – 1.8 and 50µg/ml -1.6

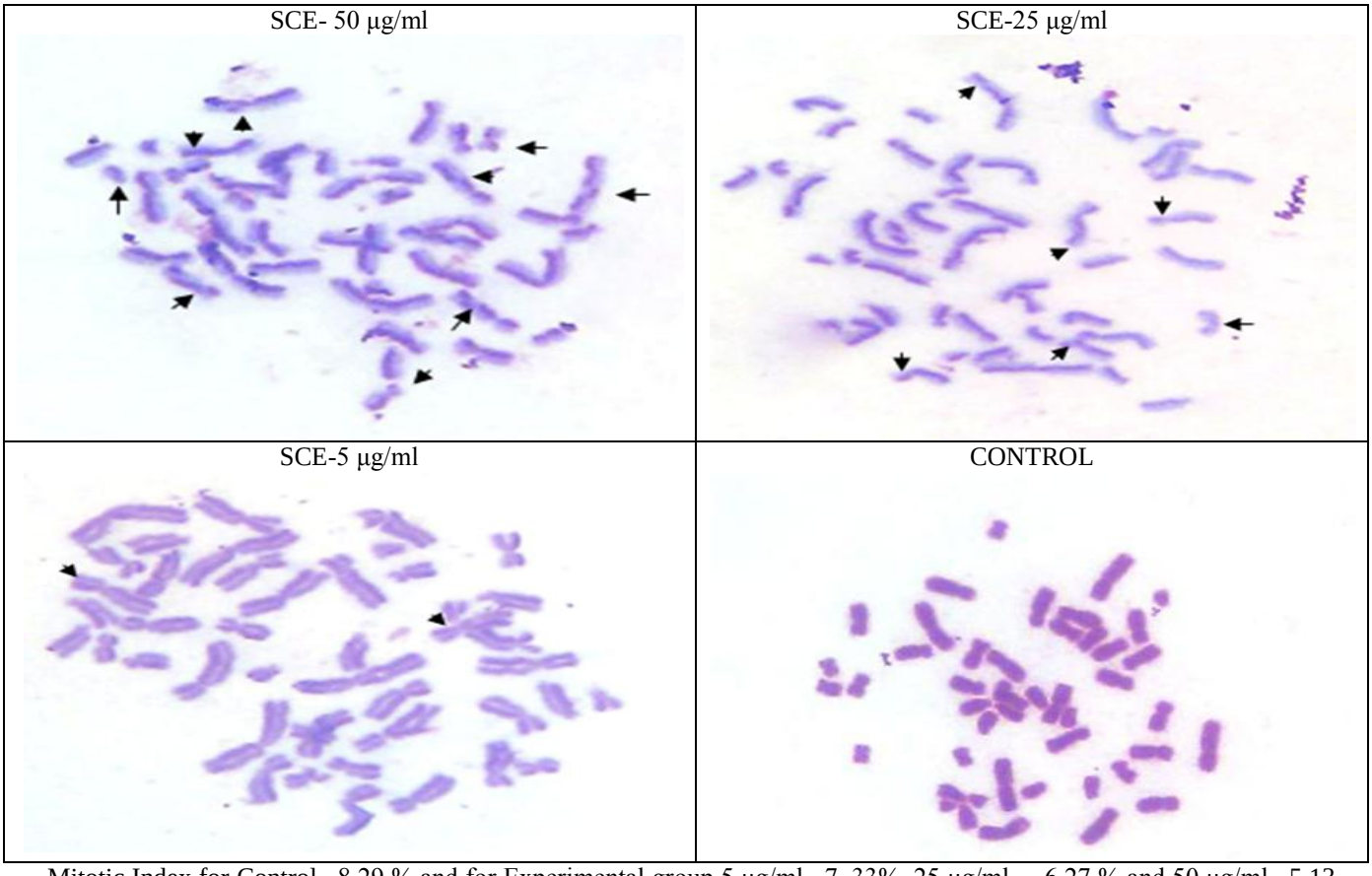


B -5µg/ml **C** -25 µg/ml **D** -50 µg/ml

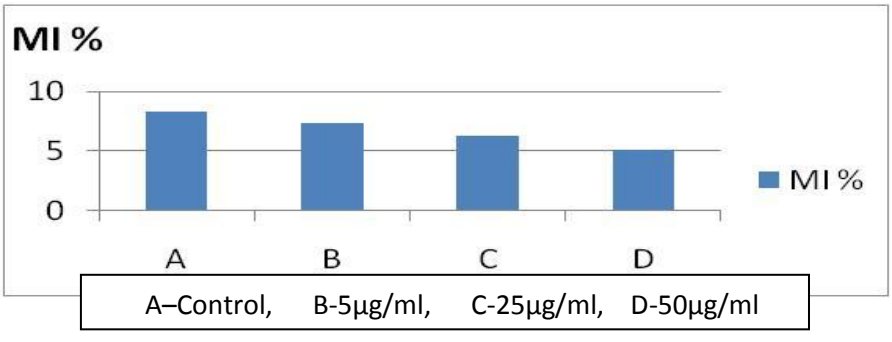
Sister Chromatid Exchange noted per cell in the control – 0 to 1, in experimental group treated with Ciprofloxacin 5 µg/ml – 0 to 1, 25 µg/ml – 0 to 4 and 50 µg/ml -0 to 6.



A – Control, B - 5µg/ml, C - 25µg/ml, D - 50µg/ml



Mitotic Index for Control– 8.29 % and for Experimental group 5 µg/ml– 7. 33%, 25 µg/ml – 6.27 % and 50 µg/ml– 5.13 %



DISCUSSION

The results obtained shows that there is a decrease in Mitotic Index and Replicative Index in 25µg/ml and 50 µg/ml of Ciprofloxacin concentration than control and 5µg/ml, but an increased incidence of Sister Chromatid Exchange was observed with an increased concentration of Ciprofloxacin than control. Decrease in Mitotic index with the increase in the concentration of the Ciprofloxacin, indicate decrease in cell division with increased cell damage. Increased incidence of Sister chromatid exchange with the increase in concentration of the Ciprofloxacin indicate increased DNA damage. Decreased Mitotic index and Replicative index and increased incidence of Sister Chromatid Exchange with increase in concentration of the Ciprofloxacin indicate the Cytotoxic and Genotoxic Effect of Ciprofloxacin. This study shows that even at lower concentration, Ciprofloxacin can exert Cytotoxic and Genotoxic effect on cultured human lymphocytes, probably due to cross reactivity with mammalian Topoisomerase ll.

CONCLUSION

Thus from our study we observed that Ciprofloxacin has Cytotoxic and Genotoxic effects on cultured human lymphocytes *in vitro.* This might be one of the reasons for various ill effects of Ciprofloxacin. Further studies need to be done including more number of healthy volunteers, with higher concentrations of Ciprofloxacin, with other assays like Micronucleus and Chromosomal aberration assay for definite conclusion about mutagenic potential of Ciprofloxacin.

ACKNOWLEDGEMENT

I gratefully acknowledge the Head of the Department of Pharmacology and Prof. Dr. S.Seethalakshmi, Prof. Dr. Darling Chellathai who helped me to carry out this research work. I am grateful to Prof. Dr.Venkatachalam.Ph.D, Human Genetics Department, SRU, for permitting to accomplish this study in cytogenetics laboratory and also thankful to Assistant Prof. Dr.Vijayalakshmi J. Ph.D, Human Genetics Department, SRU, for guidance.

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