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Evaluation of Chromosomal Aberrations Induced By Moxifloxacin.

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ABSTRACT:
Antibiotics have been reported to cause pollution in aquatic system by producing resistant genes and various chromosomal aberrations. The present study was conducted to understand the genotoxic behavior of moxifloxacin using *Allium cepa* root tip test system. The study includes Mitotic Index (MI) under the influence of moxifloxacin and observation of various Chromosomal Aberrations (CAs). The results present a concentration and time dependant behavior decrease in MI and increase in Chromosomal Aberrations (CAs). A significant ($p<0.05$) difference in MI was observed to that of control and among the types of CAs, anaphase bridge occurred most. Highest chromosomal aberrations were found at 5000µg/L at 72 hrs.

1. Introduction

Indiscriminate use of antibiotics causes pollution to ecosystem and is also harmful to human health [1-2]. Antibiotics are used for therapeutic management of infection in humans and animals [3-4]. However, discharges from pharmaceutical industries, wastewater treatment facilities (WWTF), flushing of old and out of date prescriptions, leakage from septic systems and agricultural waste storage systems are responsible for discharging of antibiotics into the environment [5]. Incomplete removal of such compounds by WWTF escalates the development of antibiotic resistant genes and hence produced super bugs [2], [6].

The occurrence of a wide range of fluoroquinolone antibiotics induce toxic effects [7], and genotoxic effects because of their ability to inhibit mammalian topoisimerase II, including induction of transit DNA strand breaks during replication, disjunction during meiosis and chromosome condensation. As these fluoroquinolones find their way in the aquatic system through effluent discharge, therefore it is important to determine the genotoxic effect of such antibiotics in natural resource [8]. Our objectives were to study the genotoxic and mitotic effects of moxifloxacin on root tip meristems.

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2. Materials and methods

2.1. Chemicals

Moxifloxacin commercialized as fine pure powder, was purchased from Yarrow Chemicals (India) with purity higher than 99%. HCl, Acetocarmine were purchased from LOBA Chemicals (India). The reagents were of AR grade and used as received. The antibiotic stock solution was prepared in distilled water and was stored at 20ºC in the refrigerator before use.

2.2. Experimental material

The genotoxic effects were evaluated by using *Allium cepa* root system, which give parallel results to in vivo cytotoxicity test. Diseased free and healthy onion bulbs were chosen for the study.

2.3. Test procedure

The dry scales and old roots were removed. Roots were grown till new roots reached about 1 cm in length. The temperature was maintained at about 25^{o}C. For each experimental group, 5 bulbs were kept. Three different concentrations of moxifloxacin antibiotic taken for the study were 50, 500 and 5000 µg/L. Before putting onion bulbs with roots in contact with their separate treatment, 5 roots on an average were collected. Next, the roots were exposed for 24 hours, (ET 24). After this, again 5 roots were collected. Hence, the bulbs with roots were kept again in their separate treatment dosages for 48 (ET48) & 72 (ET 72) hrs respectively. The root tips were collected after 24 hrs interval and processed for squash preparation. The slides were prepared immediately after each collection of roots. Control was maintained using distilled water.

2.4. Squash preparation

The roots tips were treated with 1N HCl (60^{o}C for 10 minutes). The root tips were stained with 2% acetocarmine and heated again for 10 minutes at 60^{o}C. The tips were then squashed with few drops of acetocarmine on a slide and observed under microscope at 40X and 100 X for the study of mitotic index (MI) and chromosomal aberrations (CAs).

Approximately 5000 cells/ group (Total Metaphase Observed- TMO) were investigated for aberration study. Mitotic index calculated to understand the mitodepressive effect of the drug as

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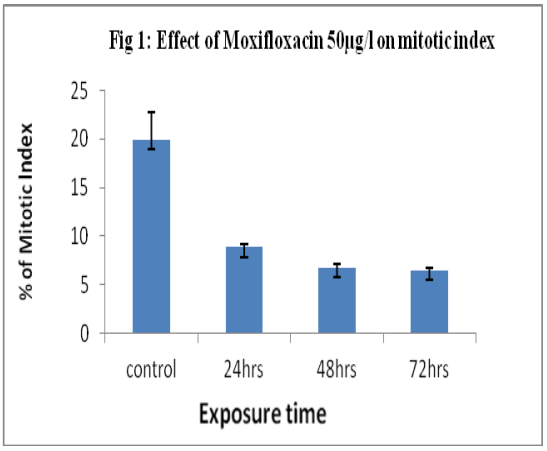
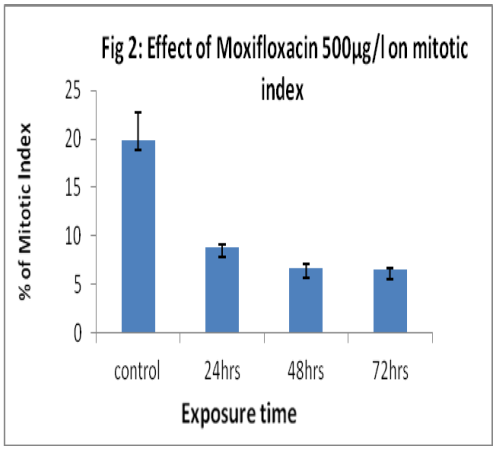
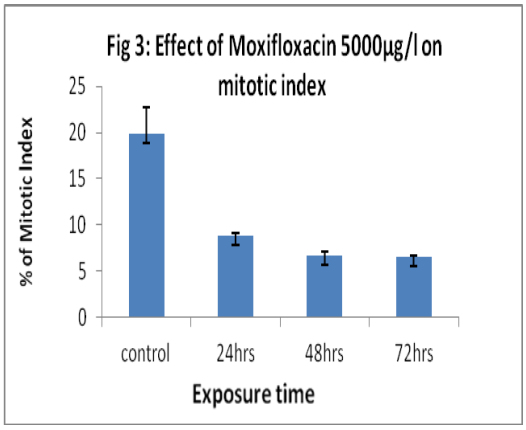
(MI = no. of dividing cells ×100/total no. of cells). 'T' test was employed at 5% significance level.

3. Result

The effect of fluoroquinolone moxifloxacin on the mitotic activity is represented in Fig. 1-3 which shows that the drug induces significant reduction in the mitotic activity in the root tip cells. Interestingly, as the time interval increased from 24 hours to 72 hours there is gradual reduction in the mitotic activity which signifies the cytotoxic effect of the drug. The study showed that there is approximately 50% reduction in MI observed started from 24 hrs with gradual decline in till 72 hrs of the study. This reduction may be accounted for the harmful effect of the drug even at the lowest level taken for the study. The significant ($p < 0.001$) reduction was found that in the samples treated with all the concentration and all the time intervals taken.







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Moxifloxacin induces varied chromosomal aberrations (anaphase bridge, multipolar anaphase with bridge, multipolar anaphase, telophase with bridge, binucleated cell, and vagrant chromosome). Table 1 shows the value of all the different types of aberrations induced by the drug. The most number of chromosomal aberrations encountered was anaphase bridge and the types of which shows the effect of the drug on the chromosomal aberrations for all the time intervals taken in the study. Highest chromosomal aberrations were found at 5000µg/L at 72 hrs time interval and the least value was observed for 50µg/L at 24 hours of the time interval. No significant difference was observed while comparing the data of the number of chromosomal aberrations observed in experimental and untreated root tip cells.

Table 1: Number of chromosomal aberrations obtained from the *Allium cepa* tests, by Moxifloxacin in different concentrations.

		24hrs			48hrs			72hrs		
Analysis	Control	50µg/l	500µg/l	5000µg/l	50µg/l	500µg/l	5000µg/l	50µg/l	500µg/l	5000µg/l
AB	9	20	16	33	24	31	37	23	33	27
MAB	1	6	8	11	8	5	14	7	8	16
MB	3	8	10	10	5	12	11	9	7	10
ML	2	16	20	28	18	16	23	15	21	29
TB	4	7	12	16	10	14	19	10	13	31
BC	1	7	10	19	5	9	20	11	9	24
VC	2	8	13	13	9	8	17	8	11	18
AD	2	10	11	12	22	11	16	10	12	19
OT	8	22	35	38	27	37	41	29	40	44
TCA	32	104	135	190	118	143	198	122	154	208
TMO	5000	5000	5000	5000	5000	5000	5000	5000	5000	5000
Mean of TCA- ±SE	0.64±0.12	2.09±0.19	2.69±0.63	3.80±0.11	2.36±0.11	2.85±0.21	3.95±0.33	2.43±0.18	3.07±0.10	4.16±0.30

AB- Anaphasic bridge, MAB- Multipolar anaphase with bridge, MB- Multipolar anaphase, ML- Metaphase with loss, TB- Telophase with bridge, BC- Binucleated cell, VC- Vagrant chromosome, AD- Adherence, OT- Others TCA- Total number of cells with alterations

4. Discussion

Chromosomal aberration assay in vitro is the potent mechanism to assess the effect of genotoxic materials. Aberrations occur after toxicity to the system [9]. *Allium cepa* was employed to study nucleotide damage through chromosome aberrations and mito- depressive assay. This test ensures the result of various end points, among all chromosomal aberration tests [10]. Researches reveal that there is a strong correlation among abnormalities of chromosomes and mutagenecity of root tips of plants and cells of mammals. *Allium cepa* roots are believed to be the initial among organisms used for cytogenetic studies to find toxicity and genotoxicity of xenobiotic [11]. *Allium cepa* was used as it is easy to handle and sensitive to environmental mutagens and can be correlated to mammalian system [12].

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The toxic effect of moxifloxacin was assessed by examining onion root meristem cells. The higher concentration of moxifloxacin was mostly found to reduce cell division. Depression in cell division index is a marking system to clarify the cytotoxic effect. Disagreement to the present study, that fluoroquinolone is genotoxic to chromosomes however, gatifloxacin induce weak clastogenic effect as compared to positive control [13]. The potential cytotoxic and genotoxic parameters such as number of chromosomal abnormalities, including anaphase with bridges, sticky chromosomes, vagrant chromosomes and multipolar anaphase were incorporated in the study. Fluoroquinolones are readily biodegradable but with low degradation rate [14]. When moxifloxacin concentrations were tested on *Allium cepa* cells to evaluate their action on the kinetic cell cycle, a decrease in mitotic indices was observed. The inhibition of mitotic activity was observed for all the doses taken into consideration. The study also gets support by the fact that norfloxacin greatly reduces the *Salmonella typhimurium* TA102 strain [15]. Purlifloxacin also induces chromosomal aberrations in mice bone marrow cells [16].

Depression in mitotic activity defines the toxicity of the chemical interaction with the plant root system. This work is supported by the reduction in MI because of ciprofloxacin [17]. If the mitotic index decrease below 22% of the control it causes fatal effects on test organisms, whereas sub lethal effects has been found if mitotic index decrease below 50% and is called cytotoxic limit value [18]. The fluoroquinolone, moxifloxacin also shows mitotic depression, which was also found to be concentration dependent. Fluoroquinolone, gatifloxcin exhibits concentration-dependant increase in incidences of chromosomal aberrations and also shows CAs with increase in time interval [7].

The most number of aberrations found were anaphasic bridges. The most number of anaphasic bridges were found at higher concentration of moxifloxacin (5000µg/L) at 72h exposure time (Table-1). This clearly states that the drug is more toxic with time. Bridge formation was also observed in the metaphase plates. The result shows a concentration and time dependant increase in the formation of bridges with significant difference ($p<0.05$) to that of control. The broken ends of the chromosomes may fused and result in formation of

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bridge [19]. The study also depicts that the drug also causes other types (OT) of aberrations during the experiment which could not be classified.

Adhesion in the proteins of the chromosome may be the reason for stickiness of chromosomes which were found in onion roots [20]. Adherence of the chromosomal elements was also observed in the experiment which is about 0.44% and is significantly higher than the control group. Patil and Bhat, 1992, were of the opinion that this adherence is due to stickiness in the chromosomal materials found in the root tip cells. Vagrant chromosome mostly moves ahead of from its chromosomal group toward poles which leads to the unequal separation of number of chromosomes in the daughter cells [21].

So, the study gave a clear indication that MI and CAs are important factor to understand the clastogenic and genotoxic effects of quinolones. Moxifloxacin induced a s significant decrease in MI when compared to control, however dose and time dependant reduction could not be observed. The CAs are accounted in the experiment but varied genetic damage shows the genotoxic effect of the drug. These cytotoxic and genotoxic effects may lead to mis-aggregation to loss of genes which can cause cell death.

5. Conclusion

It can be concluded that the exposure to moxifloxacin can cause genotoxic effect in *Allium cepa* root tip cells. Although the clastogenic and genotoxic effect of the antibiotic indicates that prolonged exposure and continuous consumption can generate a potential hazards to human health.

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