ORIGINAL ARTICLE JGEB Vol. 7, No. 1, 2009 ISSN:1687-157X

Antimutagenesis of Vitamin AD_3E Mixture to Mutations Induced by Flouroquinolone Drug Ciprofloxacin on Mice.

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ABSTRACT

Recently, considerable interest has developed regarding the presence of pharmaceutical product in the environment. Human, livestock, birds, aquatic animals, plants and different organisms have been shown to be adversely affected by drugs persisting in soil and water. The genotoxic effect is one of the serious risks. The flouroquinolones are one of the main classes of antimicrobial drugs used in the worldwide. The aim of this study was undertaken to investigate antimutagenic effect of Vitamin AD3E mixture against the genetic damage induced by ciprofloxacin (CFX) drug. The following genetic endpoints were used: 1- Cytogenetic chromosome analysis in somatic and germ cells, 2- DNA fragmentation assay in mouse spleen cells. The results obtained in this study showed that Vitamin AD3E inhibit the DNA damage induced by CFX in dose and time dependent in compared to the CFX alone. The results indicated that vitamin AD3E has antimutagenic effect against genetic damage induced by CFX drug.

Key Words: Antimutagenicity, vitamin AD_3E mixture, ciprofloxacin, DNA damage

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Journal of Genetic Engineering and Biotechnology, 2009, 7(1): 35-43

INTRODUCTION

Quinolones are currently one of the main classes of antimicrobial used worldwide. The clinical use of quinolones is not restricted to human medicine but is also widely applied in the treatment and prevention of veterinary diseases in food-producing animals and even as growth-promoting agents *(Greene and Budsberg, 1993 and Martinez et al., 2006)*. Different studies were carried out concerning the genotoxic effect of quinolones. *(Mc Queen et al., 1991; Shimada and Itoh, 1996 and Enzmann et al., 1999)*.

Ciprofloxacin (CFX) is an extended spectrum antimicrobial drug belongs to fluroquinolones (*McKellar et al., 1999*). It acts as bactericidal by altering the action of bacterial DNA gyrase, a type II topoisomerase that responsible for supercoiling of bacterial DNA *(Vancutsem et al., 1990)*. Inhibition of this activity is associated with rapid cell death in bacteria *(Hussy et al., 1986)*.

Ciprofloxacin is the ultimate reactive metabolite of enrofloxacin (EFX) converted by the enzyme cytochrome P-450 *(Vaccaro et al., 2003)*. It has shown to be mutagenic in TA102 strain of Salmonella *(Gocke, 1991)*. Positive results of CFX in human and animals in vitro *(Curry et al., 1996 and Itoh et al., 2006)* and in vivo *(Takayama et al., 1995 and Ikbal et al., 2004)* were observed. The cytotoxicity of CFX was evaluated in cultured human peripheral blood lymphocytes in patients treated with the drug in vivo *(Ikbal et al., 2004)*. *Gürbay, et al. (2005)* showed that this drug induced cytotoxicity and apoptosis in Hela cells. Also *Gürbay, et al. (2007)* observed that CFX-induced cytotoxicity in rat astrocytes.

Different studies revealed teratogenic and fetotoxic effects of CFX. *Loebstein, et al. (1998)* observed that women treated with CFX had a tendency for an increased rate of therapeutic abortions. *Channa and Janjua, (2003)* strongly suggested that CFX, given during pregnancy, causes sever liver damage in fetuses of Wistar albino rats. This finding was further supported by *Minta, et al. (2005)*.

There is general agreement that the Mediterranean diet riches with vitamins. This diet contributes to the prevention of various chronic degenerative diseases such as cardiovascular diseases and cancer *(Zhang et al., 2009)*.Vitamin AD_3E mixture was used in this study as antimutagenic agent against DNA damage induced by CFX drug. Vitamin A (VA) is one of the most important nutrients essential for normal growth and differentiation *(Emura et al., 1988)*. VA is found in liver, eggs, milk, butter, carrots, vegetables, orange and yellow fruits *(Haslett et al., 1999)*. It is vital to eye and retina function, regulates multiple biological processes, including cell proliferation, differentiation and death. So it plays critical roles in embryonic development *(Louis, 1986 and Emura et al., 1988)*. A considerable wealth of research data has been accumulated regarding the efficacy of VA as an antimutagenic *(Antunes et al., 2005 and Wang et al., 2006)* and anticarcinogenic agent *(Toma et al., 1998 and Simeone et al., 2005)*.

Vitamin A could have three mechanisms of action. First, an antioxidant action which leads VA to protect the genome against free radicals *(Antunes et al., 2005)*. Secondly, it has been shown that VA presents a selective inhibition of the mutagen metabolic activation pathway catalyzed

predominantly by hepatic microsomal cytochrome P450 dependent monooxygenase system *(Decoudu et al., 1992)*. Thirdly, VA may interact with DNA and so could protect the genome towards reactive intermediates *(Decoudu et al., 1992)*.

Vitamin D_3 (VD3) plays a major role in mammalian calcium and phosphorus homeostasis and bone health. VD_3 exerts pleiotropic effects on cell proliferation, differentiation and the immune system *(DeLuca, 2004)*. VD_3 has direct anti-inflammatory properties on microglial cells *(Lefebvre d'Hellencourt et al., 2003)*.

The biologically active metabolite of VD (1, $25(OH)_2D_3$) may play an important role in human cancer. Increased risk of breast, prostate and colon cancer have been associated with reduced serum concentration of $1,25(OH)_2D_3$ *(Studzinski and Moore, 1995)*. The antimutagenic activity of VD3 was evaluated by several authors *(Sarkar et al., 2000 and Dusso et al., 2004)*. The protection effect of VD3 may be attributed to its ability to detoxification of the endo- and xenobiotics *(Kutuzova and DeLuca, 2007)*.

Vitamin E (VE) is an essential element of human nutrition. Many of its actions are related to its antioxidant properties *(Louis, 1986)*. The antioxidant action of VE is also significant to the genetic material stability because autoxidation products of lipids and unsaturated fatty acids are highly toxic mutagenic substances *(Vaca et al., 1988)*. Many in vitro and in vivo studies have indicated a relationship between VE supplementation and reduced risk of cancer *(Albanes et al., 2000 and Kune and Watson, 2006)* and DNA damage *(Mozdarani and Salimi, 2006 and Lorenzetti et al., 2007)*.

The object of the present study was to evaluate the antimutagenic effect of vitamin AD_3E against mutations induction by CFX drug. Taking into account the possible benefit of this therapeutic drug.

MATERIALS AND METHODS

Animals:
Male white Swiss mice aged 9–12 weeks were used in all experiments. The animals were obtained from a closed random-bred colony at the National Research Centre. The mice used for any one experiment were selected from mice of similar age (±1 week) and weight (±2 g). Animals were housed in polycarbonate boxes with steel-wire tops (not more than five animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22±3°C with a relative humidity of 50±15% and a 12-h light/dark photoperiod. Food and water were provided *ad libitum*. Animals were sacrificed after treatment by cervical dislocation.

Chemicals:
Ciprofloxacin (CFX) was purchased from Amoun Pharmaceutical Co., Egypt and vitamin AD_3E mixture was purchased from Alwatanya Co., Egypt.

Treatment and cytological preparations:
Chromosome aberrations in bone marrow cells:
For analysis of bone marrow cells, mice were orally treated (using a stomach tube) with a single dose of CFX at doses of 65,130(therapeutic dose) and 260 mg/kg b.wt. Other groups of mice were given 70 and 140 mg/kg b.wt. AD_3E, simultaneously with the highest dose of CFX. Samples were taken 24 h after treatment.

For the repeated dose experiment, mice received daily oral doses of 130 mg /kg b.wt. CFX for 1 and 2 weeks (7 and 14 days). Samples were taken 24 h after the last treatment. In the repeated dose treatments, other groups of mice were given 70 and 140 mg/kg b.wt.AD_3E, simultaneously with the CFX. A negative (non-treated) and positive with 1 mg/kg b.wt. mitomycin C groups of mice was tested. In addition, another group of mice was given the oral doses of AD_3E (70 and 140 mg/kg b.wt.) for 2 weeks.

Bone marrow preparations were made according to the technique described by *Yosida and Amano (1965)*. A group of five mice was used for each treatment and 100 well-spread metaphases were analyzed per animal for scoring of different kinds of abnormalities including gaps, breaks, fragments, deletions, Robertsonian translocations, endomitosis and polyploidy metaphases.

Sister chromatid exchanges (SCEs):
For analysis of SCE's, mice were orally treated with a single dose of CFX at doses of 65,130 and 260 mg/kg b.wt. Another group of mice used as control. Samples were taken 24 h after treatment. The method described by *Allen (1982)*, for conducting in vivo SCE's induction analysis in mice was applied with some modifications. The fluorescence-photolysis Giemsa technique was used *(Perry and Wolff, 1974)*. The frequency of SCE's was recorded for each animal in 30 well spread metaphases for SCE's /cell.

Chromosome aberrations in spermatocytes:
For analysis of spermatocytes, mice were treated as in chromosome aberrations in bone marrow cells above. Chromosomal preparations from testes were made according to the technique developed by *Evans, et al. (1964)* and 100 well-spread diakinesis metaphase-I cells were analyzed per animal to assess abnormalities in five mice per group. Metaphases with univalents, chromosome breaks and/or fragments and chain (IV) were recorded.

Sperm-shape abnormalities:
Groups of five mice were orally treated with CFX daily for five consecutive days at dose levels of 65,130 and 260 mg/kg b.wt. Animals were sacrificed 35 days after the first treatment by cervical dislocation. Sperm from negative (non-treated) and positive with 1 mg/kg b.wt. mitomycin C was tested. Sperm were prepared according to the recommended method of *Wyrobek and Bruce (1978)*. The epididymides were excised and minced in 2ml physiological saline, dispersed and filtered to remove large tissue fragments. Smears were prepared and stained with 1% Eosin Y.

DNA Fragmentation Assay:
For DNA fragmentation assay CFX with the doses 130 and 260 mg/kg b.wt. were used as single doses and 130 mg/kg b.wt. for 7 and 14 days as repeated treatment. Vitamin AD_3E

at 140 mg/kg b.wt. was used with the highest single dose and repeated dose for 14 days of CFX.

DNA Fragmentation % (DPA Assay):

The colorimetric estimation of DNA content was detected according to *Perandones, et al. (1993)* with some modifications. Both supernatant and the pellet were used for DPA assay after acid extraction of DNA.

The percentage of DNA fragmentation was expressed by the formula:

$$\% \text{ DNA fragmentation} = \frac{\text{O.D. of supernatant}}{\text{O.D. of supernatant + O.D. of pellet}} \times 100$$

DNA fragmentation (agarose gel electrophoresis):

The method of DNA fragmentation was carried out according to *Perandones, et al. (1993).*

Statistical analysis:

The significance of the difference between groups and negative control and between CFX with AD_3E against CFX alone was calculated using the t-test.

RESULTS

Chromosome aberrations in bone marrow cells:

Table (1) and Figure (1) present chromosomal aberrations induced in bone marrow cells after single and repeated oral treatments with different doses of CFX. The results show that the test doses of CFX induced a statistically significant increase in the percentage of chromosomal aberrations even after excluding gaps. Such percentage was found to be dose- and time- dependent. The results in Table 1 also demonstrate that the percentage of chromosomal aberrations in bone marrow cells was significantly reduced in all groups of mice treated simultaneously with AD_3E at 70 and 140 mg/kg b.wt. and CFX at the tested dose levels.

Table (1) shows that successive treatment of mice for 14 days with CFX and vitamin AD_3E significantly reduced the percentage of chromosomal aberrations. It reached 47.90 and 56.30 % reduction in the percentage of chromosome damage after treatment with the two doses of AD_3E, respectively.

Table 1: Detailed results of chromosome aberrations induced in mouse bone - marrow treated with CFX and CFX with vitamin AD_3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations NO (mean %±S.E.)	Including gap Excluding gaps (mean %±S.E.)	II % (Excluding gaps)		Types of chromosome aberrations Gaps ..	Break and/ or Frag.	Del.	RT.	End.	Poly.	MA
Control	—	22	4.40±0.24	2.00±0.31		12	7	3	–	–	-	-
Mitomycin C	1	141	28.20±0.37**	23.0±0.31**		26	71	5	4	9	6	20
Single AD_3E	70	23	4.60±0.53	2.60±0.57		10	11	1	-	-	-	1
	140	21	4.20±0.20	2.20±0.58		10	8	3	–	–	–	–
CFX	65	39	7.80±0.37**	4.40±0.40**		17	18	4	–	–	-	-
	130	55	11.00±0.54**	6.60±0.60**		22	24	4	–	3	1	1
	260	68	13.60±0.87**	8.20±0.52**		27	27	5	–	4	1	4
CFX+AD3E	260+70	57	11.40±O.60	5.80±0.58♦	29.30	28	24	2	–	1	-	2
	260+140	47	9.40± 0.50♦♦	4.80± 0.55♦♦	41.50	23	18	2	–	3	-	1
Repeated 1 week AD_3E	70	20	4.00±0.43	2.20±0.50		9	8	1	-	-	-	2
	140	18	3.60±0.30	2.00±0.58		8	8	2	–	–	–	–
CFX	130	80	16.00±0.70**	10.80±0.86**		26	40	4	1	3	-	6
CFX+AD_3E	130+70	59	11.80±0.40♦♦	7.00±0.66♦	35.20	24	29	2	-	2	-	2
	130+140	52	10.40± 0.52♦♦	6.20± 0.58♦♦	42.60	21	27	2	-	1	-	1
2 weeks AD_3E	70	21	4.20±0.48	2.20±0.50		10	8	1	-	-	-	2
	140	22	4.40±0.40	2.00±0.58		12	7	2	–	–	–	1
CFX	130	106	21.20±0.50**	14.20±0.60**		35	55	4	1	3	1	7
CFX+AD_3E	130+70	69	13.80± 0.58♦♦	7.40± 0.50♦♦	47.90	32	27	2	-	4	1	3
	130+140	55	11.00± 0.54♦♦	6.20± 0.31♦♦	56.30	24	23	3	-	2	2	1

500 metaphases examined in five mice per treatment. RT. = Robertsonian translocation , Del.=Deletion, End.= Endomitosis, Poly.= Polyploidy MA=metaphases with more than one aberrations ** Highly significant $p < 0.01$ level (t-test) comparing to control.
♦Significant $p < 0.05$ level ♦♦Highly significant $p < 0.01$ level (t-test) comparing to treatment.

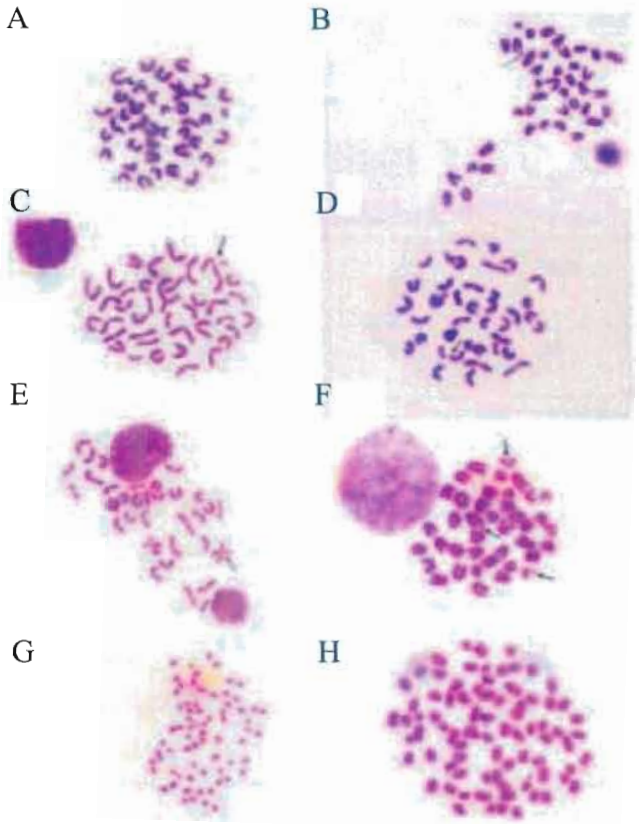

Figure 1: Metaphase plates from mouse bone marrow cells after treatment with ciprofloxacin showing, A) Gap; B) Fragment; C) Break; D) Deletion; E) Robertsonian translocation; F) Break and fragment; G) Endomitosis; H) Polyploidy.

Sister chromatid exchanges (SCEs)

Table (2) and Figure (2) show a detailed study of the effect of single oral treatment with different doses of CFX (65, 130 and 260 mg/kg b.wt.) on the induction of sister chromatid exchanges in mouse bone marrow cells 24 h. after treatment. The percentage of SCE's increased with increasing the dose of the drug. It reached 8.80±0.25 and 10.65±0.53/cell ($P<0.01$) after treatment with 130 and 260 mg/kg b.wt., respectively compared with 4.62 ± 0.40 /cell for control (Table 2).

Table 2: Frequency of sister chromatid exchanges (SCEs) in mouse bone-marrow cells treated with CFX.

Treatment and Dose (mg/kg b. wt.)	No. of different types of SCEs/chromosome			Total No. of SCEs	SCEs/Cell Mean %±SE
	Single	Double	Triple		
Control	595	49	-	693	4.62±0.40
Mitomycin C	1521	360	48	2385	15.90±0.72**
CFX					
65	663	66	5	810	5.40±0.53
130	1027	133	9	1320	8.80±0.25**
260	1272	145	12	1598	10.65±0.53**

The total number of scored metaphases is 150 (5 animals/ group)
** Highly significant $p < 0.01$ level (t-test).

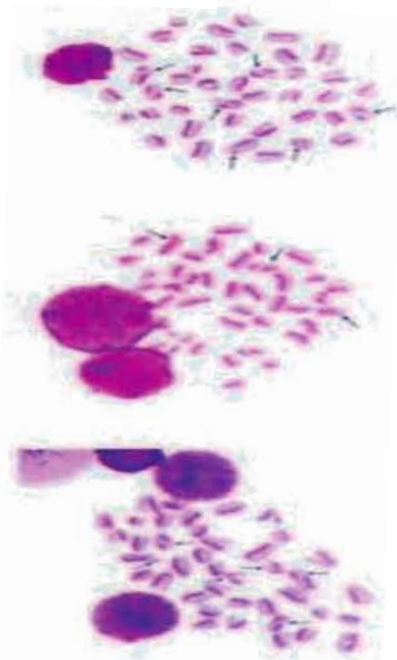

Figure 2: Metaphase plates with sister chromatid exchanges from mouse bone marrow cells after treatment with ciprofloxacin.

Chromosome aberrations in spermatocytes:

CFX at the test doses induced a significant percentage of chromosomal abnormalities in mouse spermatocytes (Table 3 and Figure 3). This percentage increased with increasing dose and with longer duration of treatment and it reached a maximum of 11.80±0.58 after repeated treatments for 2 weeks with the test dose (130 mg/kg b.wt.). It decreased to 7.00± 0.51 and 5.40± 0.50 in the CFX -treated groups that also received AD_3E at doses 70 and 140 mg/kg b.wt., respectively.

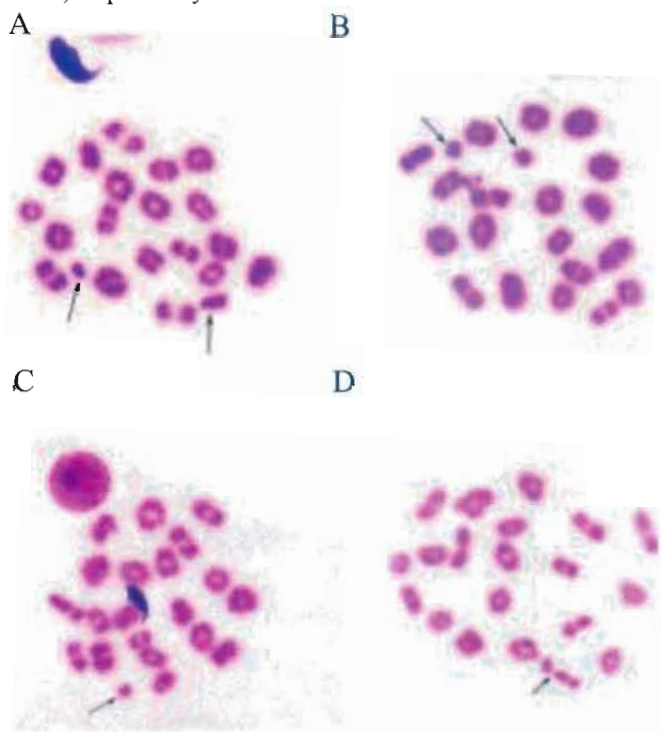

Figure 3: Diakinesis-metaphase I plates of meiosis after treatment with ciprofloxacin showing, A) X-Y univalent; B)Autosomal univalent; C) Fragment; D) Break.

Table 3: Number and mean percentage of diakinase metaphase I cells with chromosome aberrations in mouse spermatocytes treated with CFX and CFX with vitamin AD_3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations			Types of chromosome aberrations					
		NO Index	Mean %±S.E.	Inhibitory %	X-Y univalent	A.U.	X-Y univ. + A.U.	Frag. or Break	Frag. or Break + X-Y univ.	Chain IV
Control	—	15	3.00±0.31		11	3	1	–	–	-
Mitomycin C	1	108	21.6±1.16**		52	29	10	6	3	8
Single AD_3E	70	15	3.00±0.53		10	5	-	-	-	1
	140	16	3.20±0.20		10	6	-	–	–	–
CFX	65	19	3.80±0.37		14	5	-	–	-	-
	130	26	5.20±0.24**		18	7	-	1	-	-
	260	31	6.20±0.37**		19	8	1	1	1	1
CFX+AD3E	260+70	25	5.00±O.44	19.40	16	6	1	1	1	-
	260+140	23	4.60± 0.24♦	25.80	17	5	-	1	-	-
Repeated 1 week AD3E	70	17	3.40±0.40		9	8	-	-	-	-
	140	16	3.20±0.38		13	3	-	–	–	–
CFX	130	48	9.60±0.50**		30	15	-	3	-	-
CFX+AD3E	130+70	30	6.00±0.54♦♦	37.50	21	6	1	1	-	1
	130+140	24	4.80± 0.42♦♦	50.00	17	4	-	2	1	-
2 weeks AD3E	70	15	3.00±0.40		10	5	-	-	-	-
	140	17	3.40±0.42		12	5	-	–	–	–
CFX	130	59	11.80±0.58**		33	16	5	2	1	2
CFX+AD3E	130+70	35	7.00± 0.51♦♦	40.70	23	12	-	-	-	-
	130+140	27	5.40± 0.50♦♦	54.20	14	13	-	-	-	-

500 metaphases examined in five mice per treatment. X-Y univ. = X-Y univalent , A.U.= Autosomal univalent , Frag.= Fragment, ** Highly significant $p < 0.01$ level (t-test) comparing to control.
♦Significant $p < 0.05$ level. ♦♦Highly significant $p < 0.01$ level (t-test) comparing to treatment.

Morphological sperm abnormalities:

The percentage of sperm abnormalities reached 2.10, 4.50 and 5.30% after treatment with the three tested doses, respectively compared with 1.55% for the control group (Table 4). Table (4) and Figure (4) also illustrate the number and different types of sperm abnormalities after oral treatment with different doses of CFX. The dominant abnormalities found were amorphous, triangle head and coiled tail.

DNA Fragmentation Assay:

DNA Fragmentation % (DPA Assay):

Table (5) demonstrated the mean percentage of DNA fragmentation induced in mouse spleen cells after concurrent administration of antibiotic drug and antibiotic drug plus vitamin. Administration of AD_3E (140 mg/kg b.wt.) decreased the percentage of DNA fragmentation induced by the highest single dose of CFX reached 3.97 % compared to 7.12% for the group treated only with CFX. For repeated dose treatment for 2 weeks the percentage of DNA fragmentation was decreased to 4.40% ($P<0.01$) after concurrent treatment of CFX with AD_3E compared to 8.48% for CFX alone (Table 5).

DNA fragmentation (agarose gel electrophoresis):

DNA fragmentation induced by CFX assessed by agarose gel electrophoresis was decreased after simultaneous treatment with vitamin AD_3E with the dose 140 mg AD_3E/ kg b.wt compared to that treated with 260 mg CFX/kg b.wt. as single dose and 130 mg CFX/kg b.wt. as repeated dose for 14 days (Figure 5)

Table 4: Percentage of sperm abnormalities induced in male mice after oral treatment with different doses of CFX.

Treatment and doses (mg/ kg b.wt.)	No of examined sperm	Abnormal sperm		Number of sperm head abnormalities						Coiled tail
		No	Mean %±S.E.	Amorphous	Without hook	Triangle	Banana	Small	Big	
Control	5092	79	1.55± 0.40	25	12	28	2	1	-	11
Mitomycin C1	5017	784	15.60± 0.51**	185	145	177	25	9	6	237
CFX										
65	5252	110	2.10± 0.31	27	13	33	3	-	-	34
130	5350	243	4.50± 0.27**	42	31	70	8	3	1	88
260	5292	281	5.30 ± 0.31**	53	40	84	10	6	2	86

** Highly significant $p < 0.01$ level.

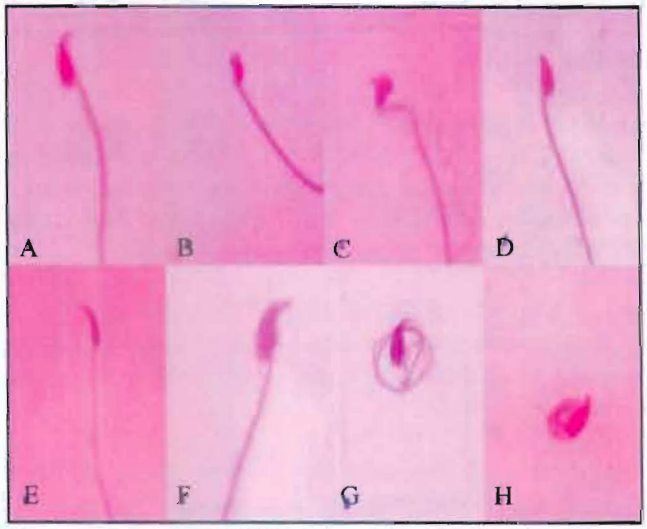

Figure 4: Sperm abnormalities induced in male mice after treatment with ciprofloxacin showing, A) normal; B) amorphous; C) Triangle; D) Without hook; E) Banana; F) Big head; G,H) Coiled tail.

Table 5: Mean percentage of DNA fragmentation induced in mouse spleen cells after concurrent treatment with CFX and CFX with vitamin AD$_3$E.

Treatment	Doses (mg/kg b.wt.)	DNA fragmentation Mean% ±S.E.	DNA fragmentation inhibition %
Control (Non-treated)	-	3.12±0.28	
Mitomycin C (positive control)	1	15.33±0.47**	
Single dose AD$_3$E	140	2.75±0.45	
CFX	130	6.20±0.28**	
	260	7.12±0.32**	
CFX+AD$_3$E	260+140	3.97±0.21♦♦	44.20
Repeated dose AD$_3$E	140×14 days	2.95±0.27	
	130×7 days	7.78±0.30**	
CFX	130×14 days	8.48±0.38**	
CFX+AD$_3$E	130×14 days+140	4.4±0.24♦♦	48.10

No of animal= 5 animal/group. ** Significant at 0.01 level (t-test) comparing to control (non-treated). ♦♦ Significant at 0.01 level (t-test) comparing to treatment.

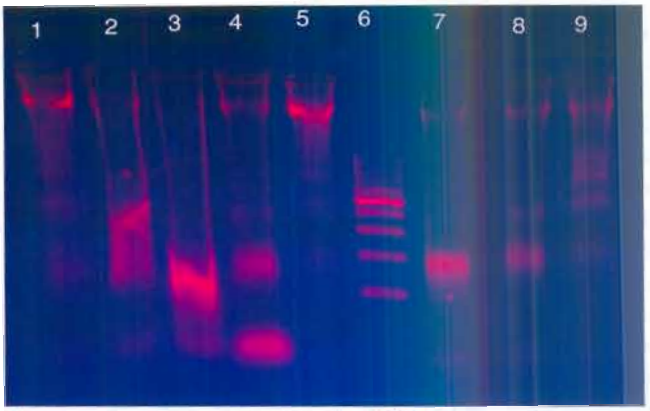

Figure 5: Relationship between the DNA fragmentation induced in mouse spleen cells by ciprofloxacin and the protective effect of vitamin AD3E against ciprofloxacin : (Lane 1: Control (non-treated); Lane 2: Positive control (Mitomycin C); Lanes 3,4: Cells treated with 130x14d and 130x7d mg ciprofloxacin/kg b.wt. respectively; Lane 5: Cells treated with 130x14d.,mg ciprofloxacin/ kg b.wt.plus140 mg AD$_3$E/kg b.wt ; Lane 6: 100 b.p. DNA ladder; Lanes 7,8: Cells treated with 260 and 130 mg ciprofloxacin/kg b.wt. respectively; Lane 9: Cells treated with 260 mg ciprofloxacin/ kg b.wt.plus140 mg AD3E/kg b.wt).

DISCUSSION

The fluoroquinolones are a class of compounds that comprise large and expanding groups of synthetic antimicrobial agents *(Van Bambeke et al., 2005)*. CFX drug belongs to flouroquinolone and is the main active metabolite of EFX *(Vaccaro et al., 2003)*. In this study, CFX induced elevation in the chromosomal aberrations in bone marrow cells and spermatocytes in dose and time dependent, comparing to the negative control. These finding run in agreement with the results of *Mukherjee, et al. (1993)* who observed that doses of 0.6, 6.0 and 20 mg CFX/kg b.wt. given intraperitoneally induced a positive dose-dependent chromosomal aberrations in mouse bone marrow cells. Basaran, et al. *(1993)* observed that administration of 20 and 200 mg CFX/kg b.wt. to rats significantly induced chromatid breakage in a dose-dependent manner. Also, CFX exerted cytotoxic effects in human fibroblast cells depending mainly on the concentration and the duration of exposure.

Also our results showed that CFX induced a significant and dose dependent elevation of SCE's in mouse bone marrow cells. These results are in agreement with those reported by *Mukherjee, et al. (1993) and Ikbal, et al. (2004)*. They found

that CFX has the ability to induce SCE's in mouse and human lymphocytes, respectively in dose dependent manner.

According to the present study, the mean percentage of sperm shape abnormalities increased by increasing the dose of CFX. *Merino and Carranza-Lira, (1995)* observed that treatment of patient with CFX did not reduce sperm quality but modified the accessory gland function. On the other hand, *King, et al. (1997)* found that this drug may decrease human sperm hyperactivation, adversely affect sperm motility and decrease rapid progression. Also, CFX at 150 mg/kg/day for 10 days induced decrease in testicular volume and sperm concentration in rats *(Demir et al., 2007)*.

The present work has shown CFX induced extensive damage in DNA of mouse spleen cells as determined by the DNA fragmentation assay. This damage was observed dose- and time-dependent. The resistance of DNA damage in spleen cells up to 14 days may be due to inhibition of Bcl-2 gene which act as antiapoptotic *(Gürbay et al., 2006)* and/or activation of some genes such as P53, Bax and caspase, which accelerates apoptosis (*Herold et al., 2002*). Our results are supported with *(Herold et al., 2002; Gürbay et al., 2006 and Lim et al., 2008)*. They observed that CFX induced inhibition of cell proliferation, DNA synthesis and apoptosis in mammalian cells in dose and time dependent.

DNA damage induced by CFX may be attributed to its ability to releasing oxygen free radical *(Gürbay et al., 2006)*. Oxygen free radical attack DNA causing mutations *(Arriaga-alba et al., 2000)*. In a trial to minimize the genotoxicity of CFX in somatic and germ cells of mice, vitamin AD_3E mixture was administered simultaneously with CFX. Our results showed that vitamin AD_3E inhibited DNA damage induced by CFX in all experimental tests. Many authors reported that vitamins A, D3 and E have the ability to inhibit the mutagenicity or carcinogenicity induced by mutagens and/or carcinogens *(Ouanes et al., 2003; 2005; Gürbay et al., 2006; 2007 and Arriaga-Alba et al., 2008)*.

The protective effect of vitamin AD_3E against DNA damage induced by CFX raises a question whether the AD_3E effect interfere with the CFX efficacy as bactericidal. *Arriaga-alba, et al. (2000)* observed that the in vitro bactericidal effect of quinolones was not altered by β-carotene, which is free oxygen radical scavenger. However, the bactericidal effect of quinolones was due to inhibition of DNA gyrase enzyme (responsible for supercoiling of bacterial DNA) *(Vancutsem et al., 1990)*. According to the observation of *Arriaga-alba, et al. (2000)*, we can presume that using vitamin AD_3E in this study, which is a free oxygen radical scavenger and/ or detoxification of endo- and xenobiotics *(Louis, 1986; De Flora et al., 1999 and Kutuzova and Deluca, 2007)* may inhibit the power of CFX to induce genetic damage without interfering with its capacity as bactericidal.

CONCLUSION

the present work indicated that the antimicrobial drug CFX has a mutagenic effect in somatic and germ cells of mice. Vitamin AD_3E mixture might be a good alternative to reduce genotoxic risk associated with quinolones therapy. Further studies need to be conducted in order to determine if the vitamin AD_3E can effectively inhibit genetic damage induced by CFX drug without effect on its action as bactericidal.

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