Antimutagenesis of vitamin AD3E 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 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. [Nature and Science 2009; 7(12): 61-71]. (ISSN: 1545-0740).

Key words: Antimutagenicity, Vitamin AD3E, Ciprofloxacin, DNA damage.

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; Martinez *et al,* 2006**). Different studies were carried out concerning the genotoxic effect of quinolones. **(McQueen *et al,* 1991; Shimada and Itoh 1996; 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).**

CFX is the ultimate reactive metabolite of enrofloxacin (EFX) converted by the cytochrome P-450 enzymes (**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; Itoh *et al,* 2006)** and *in vivo* **(Takayama *et al,* 1995**; **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 AD3E 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; Emura *et al,* 1988).**

Table (1): Detailed results of chromosome aberrations induced in mouse bone - marrow treated with CFX and CFX with vitamin AD3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations NO	Including gap (mean %±S.E.)	Excluding gaps (mean %±S.E.)	II % (Excluding gaps)	Types of chromosome aberrations: Gaps	Breakand// or Frag	Del.	RT.	End.	Poly.	MA.
Control	—	22	4.40±0.24	2.00±0.31		12	7	3	–	–	-	-
MMC	1	141	28.20±0.37**	23.0±0.31**		26	71	5	4	9	6	20
Single AD3E	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 AD3E	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+AD3E	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 AD3E	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**	4.20±0.60**		35	55	4	1	3	1	7
CFX+AD3E	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.

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
	S	D	T		
Control	595	49	-	693	4.62±0.40
MMC 1	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) S= Single D= Double T= Triple
**** Highly significant *p* < 0.01 level (t-test).**

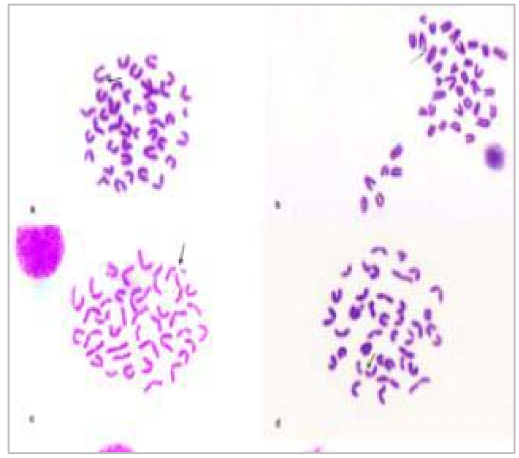

Fig. (1): Metaphase plates from mouse bone marrow cells after treatment with ciprofloxacin showing, a) Gap; b) Fragment; c) Break; d) Deletion.

A considerable wealth of research data has been accumulated regarding the efficacy of VA as an antimutagenic **(Antunes *et al,* 2005; Wang *et al,* 2006)** and anticarcinogenic agent **(Toma *et al,* 1998; 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; 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; Kune and Watson, 2006)** and DNA damage **(Mozdarani and Salimi, 2006**; **Lorenzetti *et al,* 2007).**

The object of the present study was undertaken to evaluate the antimutagenic effect of vitamin AD3E 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.

Table (3): Number and mean percentage of diakinase metaphase I cells with chromosome aberrations in mouse spermatocytes treated with CFX and CFX with vitamin AD3E.

Treatment	Doses (mg/kg b.wt.)	Total abnormal aberrations			Types of chromosome aberrations					
		NO	Mean %±S.E.	Inhibitory Index %	X-Yuniv	AU	X-Yuniv + AU	Frag or Break	Frag or Break + X-Yuniv	Chain (IV)
Control	—	15	3.00±0.31		11	3	1	–	–	-
MMC	1	108	21.6±1.16**		52	29	10	6	3	8
Single										
AD3E	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.

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

Treatment and doses (mg/kg bw)	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
MMC 1	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**.

Chemicals

Ciprofloxacin (CFX) was purchased from Amoun Pharmaceutical Co., Egypt and vitamin AD3E 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.AD3E, 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.AD3E, 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 AD3E (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.

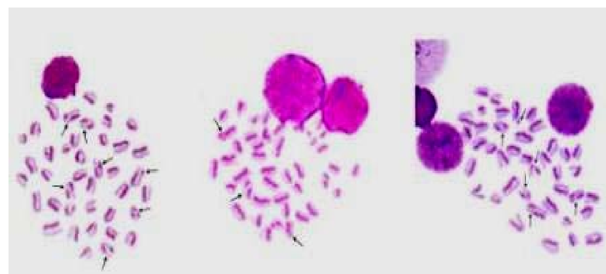

Fig (2): Metaphase plates with sister chromatid exchanges from mouse bone marrow cells after treatment with ciprofloxacin.

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 AD3E at 140 mg/kg b.wt. was used with the highest single dose and repeated dose for 14 days of CFX.

1- 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: % DNA fragmentation

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

2- 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 AD3E against CFX alone was calculated using the t-test.

Results

Chromosome aberrations in bone marrow cells.

Table (1) and Fig.(1) present chromosomal aberrations induced in bone marrow cells after single and repeated oral treatments with different doses of CFX. The results showed that the tested 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 AD3E at 70 and 140 mg/kg b.wt. and CFX at the tested dose levels.

Table (5): Mean percentage of DNA fragmentation induced in mouse spleen cells after concurrent treatment with CFX and CFX plus vitamin AD_3E

Treatment	Doses (mg/kg b.wt.)	DNA fragmentation Mean% ±S.E.	DNA fragmentation Inhibition %
Control (Non-treated)	-	3.12±0.28	
MMC (positive control)	1	15.33±0.47**	
Single dose			
AD_3E	140	2.75±0.45	
CFX	130	6.20±0.28**	
	260	7.12±0.32**	
CFX+AD_3E	260+140	3.97±0.21♦♦	44.20
Repeated dose			
AD_3E	140×14 days	2.95±0.27	
CFX	130×7 days	7.78±0.30**	
	130×14 days	8.48±0.38**	
CFX+AD_3E	130×14days+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)

Table 1 show 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.

Sister chromatid exchanges (SCEs).

Table (2) and Fig. (2) showed 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).

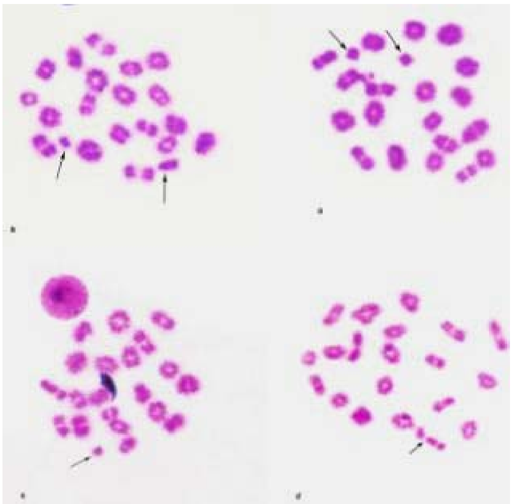

Fig (3): Diakinesis-metaphase I plates of meiosis after treatment with ciprofloxacin showing, a) X-Y univalent; b)Autosomal univalent; c) Fragment; d) Break.

Chromosome aberrations in spermatocytes.

CFX at the tested doses induced a significant percentage of chromosomal abnormalities in mouse spermatocytes (Table 3 and Fig. 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 tested 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.

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 Fig. (4) also illustrates 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.

1- 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 with 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).

2-DNA fragmentation (agarose gel electrophoresis).

DNA fragmentation induced by CFX assessed by agarose gel electrophoresis was decreased after simultaneous treatment 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 (Fig. 5) **.**

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 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; 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 manner.

DNA damage induced by CFX may be attributed to its ability to releasing oxygen free radicals **(Gürbay *et al,* 2006).** Oxygen free radicals 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 AD3E 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**; **Arriaga-Alba *et al,* 2008**).

The protective effect of vitamin AD3E against DNA damage induced by CFX raises a question whether the AD3E 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 AD3E in this study, which is a free oxygen radical scavenger and/or detoxification of endo- and xenobiotics **(Louis, 1986; De Flora *et al.*, 1999; Kutuzova and Deluca, 2007)** may inhibit the power of CFX to induce genetic damage without interfering with its capacity as bactericidal.

In 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 AD3E can effectively inhibit genetic damage induced by CFX drug without effect on its action as bactericidal.

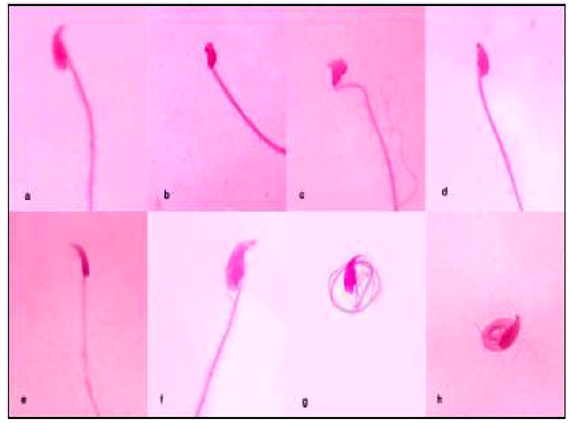

Fig (4): Sperm abnormalities induced in male mice after treatment with ciprofloxacin showing, a) normal; b) amorphous; c) Triangle; d) Without hook; e) Banana; f) Big head; g,h) Coiled tail.

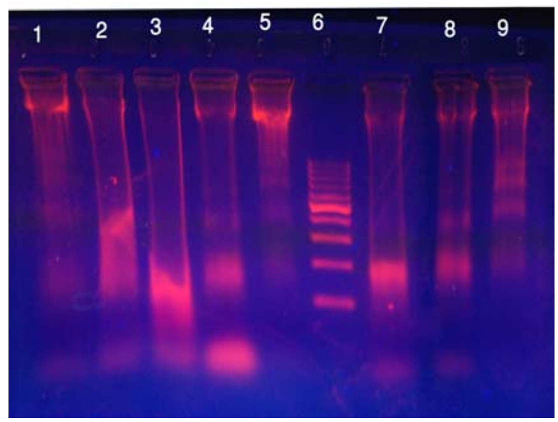

Fig. (5): Relationship between the DNA fragmentation induced in mouse spleen cells by ciprofloxacin and the protective effect of vitamin AD3E against ciprofloxacin : [**Lane 1:** Control (non-treated); **Lane 2:** Positive control (Mitomycin C); **Lanes 3,4:** Cells treated with 130x14d and 130x7d mg ciprofloxacin/kg b.wt. respectively; **Lane 5:** Cells treated with 130x14d.,mg ciprofloxacin/kg b.wt. with 140 mg AD3E/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. with 140 mg AD3E/kg b.wt].

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References:

[1] Albanes D, Malila N, Taylor PR, Huttunen JK, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Barrett MJ, Pietinen P, Hartman TJ, Sipponen P, Lewin K, Teerenhovi L, Hietanen P, Tangrea JA, Virtanen M, Heinonen OP. Effects of supplemental alpha-tocopherol and beta-carotene on colorectal cancer: results from a controlled trial (Finland). Cancer Causes Control 2000; 11: 197-205.

[2] Allen JW. A method for conducting in vivo SCE induction analysis in mice. Genetic Toxicology Division US Environ Protection Agency Research Triangle Park North Carolina 1982; 27711.

[3] Antunes LMG, Pascoala LM, Bianchib MLP, Diasa FL. Evaluation of the clastogenicity and anticlastogenicity of the carotenoid bixin in human lymphocyte cultures. Mut Res Genet Toxicol Environ Mutagen 2005; 585(1-2):113-119.

[4] Arriaga-Alba M, Rivera-Sánchez R, Parra-Cervantes G, Barron-Moreno F, Flores-Paz R , Elbia García-Jiménez E. Antimutagenesis of b-carotene to mutations induced by quinolone on Salmonella typhimurium. Arch Med Res 2000;31:156–161.

[5] Arriaga-Alba M, Rivera-Sanchez R, Ruiz-Perez N, Sanchez-Navarrete J, Flores-Paz R, Montoya EA , Hicks GJJ. Comparative study of the antimutagenic properties of vitamin C and E on mutations induced by norfloxacin. BMC Pharmacol 2008; 8(1), 2.

[6] Basaran A, Erol K, Basaran N, Gunes HV, Acikalin E, Timuralp G, Degirmenci I, Cakmak EA, Tomatir AG. Effects of ciprofloxacin on chromosomes, and hepatic and renal functions in rats. Chemother 1993; 39(3): 182-188.

[7] Channa MA, Janjua MZ. Effects of ciprofloxacin on foetal hepatocytes. J Pak Med Assoc 2003; 53(10): 448-450.

[8] Curry PT, Kropko ML, Garvin JR, Fielder RD, Theiss JC. *In vitro* induction of micronuclei and chromosome aberrations by quinolones: possible mechanism. Mut Res 1996; 352:143-150.

[9] Decoudu S, Cassand P, Daubèze M, Frayssinet C , Narbonne JF. Effect of vitamin A dietary intake on *in vitro* and *in vivo* activation of aflatoxin B1. Mut Res 1992; 269: 269-278.

[10] De Flora S, Bagnasco M, Vainio H. Modulation of genotoxic and related effects by carotenoids and vitamin A in experimental models: mechanistic issues. Mutagen 1999; 14:153–172.

[11] DeLuca H. Overview of general physiologic features and functions of vitamin D. Am J Clin Natr 80(Suppl) 2004; 1689S-1696S.

[12] Demir A, Turker P, Onol FF, Sirvanci S, Findik A , Tarcan T. Effect of experimentally induced Escherichia coli epididymo-orchitis and ciprofloxacin treatment on rat spermatogenesis. Int J Urol 2007; 14(3): 268-272.

[13] Domagala JM. Structure-activity and structure-side effect relationships for the quinolone antibacterials. J Antimicrob Chemother 1994;33: 685-706.

[14] Dusso AS, Thadhani R, Slatopolsky E. Vitamin D receptor and analogs. Semin Nephrol 2004; 24:10-16.

[15] Emura M, Mohr U, Riebe M, Aufderheide M , Dungworth DL. Regulation of growth and differentiation by vitamin A in a cloned fetal lung epithelial cell line cultured on collagen gel in hormone-supplemented medium, in vitro. Cell Dev. Biol 1988; 24: 639-648.

[16] Enzmann H, Wiemann C, Ahr HJ , Schlüter G. Damage to mitochondrial DNA induced by the quinolone Bay y 3118 in embryonic turkey liver. Mut Res 1999;425(2): 213-224.

[17] Evans EP, Breckon G, Ford CE. An air drying method for meiotic preparation from mammalian testes. Cytogenetics 1964; 3: 289–294.

[18] Gocke E. Mechanism of quinolone mutagenicity in bacteria. Mut Res 1991;248:135-143.

[19] Greene CE , Budsberg SC. Veterinary use of quinolones. In: Hopper, D.C. and Wolfson, J.S.(Eds), Quinolone antimicrobial agents, 2nd ed American society for microbiology Washington DC 1993; pp: 474-488.

[20] Gürbay A ,Gonthier B, Barret L, Favier A , Hincal F. Cytotoxic effect of ciprofloxacin in primary culture of rat astrocytes and protection by vitamin E. Toxicol 2007;229: 54-61.

[21] Gürbay A, Gonthier B, Signorini-Allibe N, Barret L, Favier A , Hincal F. Ciprofloxacin-induced DNA damage in primary culture of rat astrocytes and protection by vitamin E. Neurotoxicol 2006;27: 6-10.

[22] Gürbay A, Osman M, Favier A, Hincal F. Ciprofloxacin-induced cytotoxicity and apoptosis in HeLa cells. Toxicol Mech Meth 2005; 15:339-342.

[23] Haslett C, Chilvers ER, Hunter JAA , Boon NA. Davidson's principles and practice of medicine. 18th ed 1999; pp: 67; 519, Harcourt Publishers Limited. U.K.

[24] Herold C, Ocker M, Ganslmayer M, Gerauer H, Hahn EG , Schuppan D. Ciprofloxacin induced apoptosis and inhibits proliferation of human colorectal carcinoma cells. Br J Cancer 2002; 86:443-448.

[25] Hussy P, Maass G, Tummler B. effect of 4-quinolones and novobiocin on calf thymus DNA polymerase alpha primase complex, topoisomerase I and II, and growth of mammalian lymphoblasts. Antimicrob. Agent Chemotherap 1986; 29: 1073-1078.

[26] Ikbal M, Doğan H, Odabaş H , Pirim I. Genotoxic evaluation of the antibacterial drug, ciprofloxacin, in cultured lymphocytes of patient with urinary tract infection. Turk J Med Sci 2004 ; 34: 309-313.

[27] Itoh T, Mitsumori K, Kawaguchi S, Sasaki YF. Genotoxic potential of quinolone antimicrobials in

the *in vitro* comet assay and micronucleus test. Mut Res 2006; 603(2): 135-144.

[28] King K, Chan PJ, Patton WC, King A. Antibiotics: Effect on cryopreserved-thawed human sperm motality *in vitro*. Fertil Steril 1997; 67(6): 1146-1151.

[29] Kune G, Watson L. Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B_6, B_{12}, C, E, selenium, and lycopene. Nutr Cancer 2006; 56(1): 11-21.

[30] Kutuzova GD, DeLuca HF. 1, 25-Dihydroxyvitamin D_3 regulates genes responsible for detoxification in intestine. Toxicol Appl Pharm 2007; 218: 37-44.

[31] Lefebvre d'Hellencourt C, Montero-Menei CN, Bernard R , Couez D. Vitamin D_3 inhibits proinflammatory cytokines and nitric oxide production by the EOC_{13} microglial cell line. J Neurosci Res 2003; 71: 575–582.

[32] Lim S ,Hossain MA, Park J, Choi SH , Kim G. The effect of enrofloxacin on canine tendon cells and chondrocytes proliferation in vitro. Vet Res Commun 2008; 32(3): 243-253.

[33] Loebstein R, Addis A, Ho E, Andreou R, Sage S, Donnenfeld AE, Schick B, Bonati M, Moretti M, Lalkin A, Pastuszak A, Koren G. Pregnancy outcome following gastrointestinal exposure to fluoroquinolones: a multicenter prospective controlled study. Antimicrob Agents Chemother 1998; 42(6): 1336-1339.

[34] Lorenzetti F, Dambos M, Castro M, Ribeiro ML, Miranda DD, Ortiz V. Influence of oxidative stress and alpha tocopherol supplementation on urothelial cells of the urinary bladder in ovariectomised rats. Int Urogynecol J Pelvic Floor Dysfunct 2007; 18(11): 1351-1356.

[35] Louis St. Drugs facts and comparisons, In: Div., JB (Ed.), Facts and comparisons Lippincott co USA 1986; pp: 6-24.

[36] Martinez M, McDermott P, Walker R. Pharmacology of fluoroquinolones: A perspective for the use in domestic animals. Vet J 2006; 172: 10-28.

[37] McKellar QA, Gibson IF, Monteiro A , Bregante M. Pharmacokinetics of enrofloxacin and danofloxacin in plasma, inflammatory exudates and bronchial secretions of calves following subcutaneous administration. Antimicrob Agents Chemotherap 1999; 43: 1988-1992.

[38] McQueen CA, Way BM, Queener SM, Schlüter G, Williams GM. Study of potential *in vitro* and *in vivo* genotoxicity in hepatocytes of quinolone antibiotics. Toxicol Appl Pharm 1991; 111(2): 255-262.

[39] Merino G, Carranza-Lira S. Infection and male infertility: effect of different antibiotic regimens on semen quality. Arch Androl 1995; 35(3): 209-212.

[40] Minta M, Wilk I, Zmudzki J. Inhibition of cell proliferation by quinolones in micromass cultures of rat embryonic limb bud and midbrain cells. Toxicol In Vitro 2005; 19: 915-919.

[41] Mozdarani H, Salimi M. Numerical chromosome abnormalities in 8-cell embryos generated from gamma-irradiated male mice in the absence and presence of vitamin E. Int J Radiat Biol 2006; 82(11): 817-822.

[42] Mukherjee A, Sen S, Agarwal K. Ciprofloxacin: mammalian DNA topoisomerase type II poison *in vivo*. Mut Res 1993; 301: 87-92.

[43] Ouanes Z, Abid S, Ayed I, Anane R, Mobio T, Creppy EE, Becha H. Induction of micronuclei by zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of vitamin E. Mut Res 2003; 538: 63-70.

[44] Ouanes Z, Ayed-Boussema I, Baati T, Creppy EE , Bacha H. Zearalenone induces chromosome aberrations in mouse bone marrow: preventive effect of 17β-estradiol, progesterone and vitamin E. Mut Res 2005; 565: 139-149.

[45] Perandones CE, Illera VA, Peckham D, Stunz LL, Ashman RF. Regulation of apoptosis *in vitro* in mature murine spleen T cells. J Immunology 1993; 151: 3521-3529.

[46] Perry P , Wolff S. New Giemsa method for the differential staining of sister chromatids. Nature (London) 1974; 251: 156-158.

[47] Sarkar A, Saha BK, Basak R, Mukhopadhyay I, Karmakar R , Chatterjee M. Anticlastogenic potential of 1α,25-dihydroxyvitamin D_3 in murine lymphoma. Cancer letters 2000; 150(1): 1-13.

[48] Shimada H, Itoh S. Effects of new quinolone antibacterial agents on mammalian chromosomes. J Toxicol Environ Health 1996: 47(2): 115-123.

[49] Simeone AM, Deng CX, Kelloff GJ, Steele VE, Johnson MM, Tari AM. N-(4-Hydroxyphenyl) retinamide is more potent than other phenylretinamides in inhibiting the growth of BRCA1-mutated breast cancer cells. Carcinogenesis 2005; 26: 1000–1007.

[50] Studzinski GP, Moore DC. Sunlight: can it prevent as well as cause cancer. Cancer Res 1995; 55:4014-4022.

[51] Takayama S, Hirohashi M, Kato M, Shimada H. Toxicity of quinolone antimicrobial agents. J Toxicol Environ Health 1995; 45: 1-45.

[52] Toma S, Isnardi L, Riccardi L , Bollag W. Induction of apoptosis in MCF-7 breast carcinoma cell line by RAR and RXR selective retinoids. Anticancer Res 1998; 18: 935–942.

[53] Vaca CE, Wilhelm J, Harms-Rindahl M. interaction of lipid peroxidation products with DNA. A review. Mut Res 1988;195: 137-149.

[54] Vaccaro E, Giorgi M, Longo V, Mengozzi G , Gervasi PG. Inhibition of cytochrome P450 enzymes by enrofloxacin in the sea bass (Dicentrarchus labrax). Aquatic Toxicol 2003; 62: 27-33.

[55] Van Bambeke F, Michot JM ,Van Eldere J, Tulkens PM. Quinolones in 2005: an update. Europ Soc Clin Microbiol Infect Dis 2005; 11: 256-280.
[56] Vancutsem PM, Babish GJ, Schwark WS. The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet 1990; 80: 173-186.
[57] Wang M, Tsao R, Zhang S, Dong Z, Yang R, Gong J , Pei Y. Antioxidant activity, mutagenicity /anti-mutagenicity, and clastogenicity /anti-clastogenicity of lutein from marigold flowers. Food Chem Toxicol 2006; 44(9): 1522-1529.
[58] Wyrobek AJ, Bruce WR. The induction of sperm-shape abnormalities in mice and humans, in: A. Hollaender, F.J. de Serres (Eds.), Principles and Methods for their Detection, vol. 5, Plenum Press, New York, 1978; pp: 257–285.
[59] Yosida TH, Amano K. Autosomal polymorphism in laboratory bred and wild Norway rats, Rattus norvegicus. Misima Chromosoma 1965; 16: 658–667.
[60] Zhang X , Cao J, Jiang L, Geng C , Zhong L. Protective effect of hydroxytyrosol against acrylamide-induced cytotoxicity and DNA damage in HepG2 cells. Mut Res 2009; 664: 64-68.

10/30/2009