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HEPATO-RENAL, HAEMATOLOGICAL., AND CYTOGENETIC EFFECTS OF DANOFLOXACIN

By

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SUMMARY

This experiment was carried out on 24 guinea pigs and 54 male rats. Both rats and guinea pigs were divided into 3 equal groups. Animals in group 1 served as control. Those in second and third groups were injected i.m. with danofloxacin in doses of 5 and 10 mg/kg b. wt. respectively for 5 consecutive days.

The obtained results revealed that danofloxacin (10 mg/kg b. wt. i. m.) after the 5th day post dosing induced significant decrease in bile flow in both rats and guinea pigs. Serum levels of alanine aminotransferase (ALT), urea and creatinine were significantly elevated after the 1st and 5th days post dosing at the two dose levels. Furthermore the values of aspartate aminotransferase (AST) were significantly increased after 1st day post dosing of therapy. Serum glucose and calcium levels were insignificantly changed after drug administration.

Danofloxacin (5 mg/kg b. wt. i. m.) significantly decreased packed cell volume (PCV%) and red blood corpuscles (RBCs) count after 1st day post dosing. Meanwhile, it evoked a significant decrease in haemoglobin (Hb) contents , PCV% and RBCs count after 1st day post dosing at the dose level of 10 mg/kg b. wt. i. m.

Danofloxacin has no effect on the motility of isolated rabbit duodenum and guinea pig ileum.

Concerning the genotoxic effect of the drug on bone marrow cells, the results revealed that danofloxacin has no significant effect on the rate of mitotic division at a dose level of 5 mg/kg b. wt. However, it produced a highly significant decrease in the rate of mitosis at a dose of 10 mg/kg b. wt. Moreover. The drug induced a significant increase of structural chromosomal aberrations. The percentage of aberrations were 18.4 and 26.6 for 5 and 10 mg/kg b. wt. as compared with 2% for control non treated group. The structural chromosomal aberrations induced by danofloxacin were fragments, deletion, ring chromosome, gaps, breaks and chromosomal stickiness.

INTRODUCTION

Fluoroquinolone antibacterial agents are widely used in the clinical field because of their excellent antibacterial activity, wide spectrum and high bioavailability. Fluoroquinolones have a bactericidal mode of action affecting the DNA gyrase enzyme (Wolfson and Hooper, 1985) and they have been used in cattle, swine, turkey and poultry for control of enteric and respiratory infections (Masuda et al., 1996 and Mulazimoglu et al., 1996).

There is continued interest in the development of new quinolones in order to improve antibacterial activity, overcome bacterial resistance or diminish toxicity. Danofloxacin is a novel synthetic fluoroquinolone which has been developed exclusively for veterinary use (Mann, 1989 and Tanner et al., 1993).

Fluoroquinolones have been reported to induce hazard effects on the different organs as discussed by many authors (Hanafy, 1993; El-Ashmawy et al., 1994 and Kashida and Kato, 1997). However, no enough available data was obtained about the effect of danofloxacin on the bile flow, chromosome structures and some biochemical and haematological parameters.

Many antibiotics have been reported to induce different forms of structural and numerical types of chromosomal aberrations. Maha and Hanafy (1992) and Hemeda (1994) demonstrated that rifampicin and chloramphenicol induced different types of chromosomal aberrations. The aim of the study is to throw light on the effect of danofoxcin on bile flow and some biochemical and haematological parameters as well as its genotoxic effect on bone marrow cells of rats.

MATERIALS AND METHODS

Animals:

Twenty four varicoloured guinea pigs weighing 500-700 g each and 54 clinically healthy mature rats weighing 170-220 g each were used. Food and water were provided ad libitum.

Drugs:

A commercillay available formulation of danofloxacin (Advocin, solution of 25 mg/ml, Pfizer Co., USA) was used.

Experimental design:

Both rats and guinea pigs were divided into 3 equal groups each of 18 and 8 animals respectively. Those in group 1 were injected i. m. with 0.25 ml saline and served as control. Animals of the second and third groups were injected i. m. with danofloxacin at doses of 5 and 10 mg/kg b. wt. respectively for 5 days (the dose was estimated according to Paget and Barnes (1964) representing therapeutic and double therapeutic levels).

Twenty hours and 5 days after last injection, 4 animals (guinea pigs and rats) from each group were anaesthetized by ethyl carbamate (urethane, BDH) at a dose of 1.5 g/kg i. p. and the technique for studying the bile flow was performed according to the method described by Hoyt and Larson (1989). Flow was expressed as microliters per minute per gram of liver. At the same periods 5 rats from each group were anaesthetized with

light ether anaesthesia and blood was collected for haematological examination, erythrocyte and leucocytic counts were performed using the Improved Neubaur method (Schalm, 1975), haemoglobin content was estimated as cyanomethaemoglobin after Crosby et al., (1954) and haematocrit value was determined by the microhaematocrit technique (Schalm, 1975).

Serum samples were obtained and stored frozen at -20°C until assayed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after Reitman and Frankel (1957), creatinine (Jackson, 1975), urea (Patton and Crough, 1977). glucose (Siest et al., 1981) and calcium (Tietz, 1970).

The effect of danofloxacin on the intestinal smooth muscles contraction was studied on the rabbit duodenum and guinea pig ileum according to the method described by Magnus (1904) using Tyrod's solution at 37°C.

Chromosomal preparations were made from the bone marrow of the rats following the method described by Macgregor and Varely (1983) as follows. Five rats from each group 24 hours after the last injection were injected i. p. with colchicine (1 mg/kg), then sacrificed after 1.5-2 hours. The bone marrow was dissected out and minced with a small scissors, then immersed for 15 minutes in phosphate buffer saline. The treated bone marrow was then suspended in 0.075% KCI solution for about 25 minutes at 37°C and centrifugation was performed at 800 r. p. m. for 8 minutes. Fixation of the sediment was made several times using a mixture of methanol and glacial acetic acid (3:1), the precipitate cells were dropped on a clean previously cooled slides, then air dried and stained with Gimsa stain (4%) in phosphate buffer.

About 100 well spread metaphase figures from each animal were examined for detection of different types of chromosomal aberrations under oil immersion lens (X 1600). The mitotic index was calculated according to Brusick (1980) as follows:

$$\frac{\text{Number of divided cells}}{\text{Number of divided and non divided cells}} \times 100$$

Data were statistically analyzed according to Snedecor and Cochran (1980).

Moreover, differences between control and treated groups in frequency of mitosis , rate of chromosomal aberrations and incidence of different types of chromosomal aberrations were assessed using the Chi-Square test of the Epi-Info computer package (Epi-Info, 1994).

RESULTS

In the present study, the effect of administration fo danofloxacin for 5 days at doses of 5 or 10 mg/kg b. wt. i. m. on bile flow in rats and guinea pigs, some haematological and biochemical parameters and chromosomal structures were investigated in rats.

1. Bile flow:

The obtained data showed that danofloxacin (10 mg/kg b. wt. i.m.) after 5th day post dosing induced significant ($P<0.05$) decrease in bile flow in both rats and guinea pigs (Table 1). Meanwhile, the administration of danofloxacin at a dose of 5 mg/kg b. wt. i. m. induced insignificant ($P>0.05$) decrease in bile flow in both rats and guinea pigs after the 1st and 5th days post dosing (Table 1).

2. Biochemical findings:

Statistical analysis of the obtained data showed that there was a significant ($P<0.05$) increase in serum levels of ALT, urea and creatimine after 1st and 5th days post dosing at both dose levels (Table 2). Furthermore the values of AST were significatly increased after 1st day post dosing.

Table (1) Effect of danofloxacin (Advocin) on bile flow in rats and guinea pigs. Values are expressed as mean ±S. E. (n=4).

Species	Time post dosing	Control µl/ min/g liver	Treated	
			5 mg/kg/b.wt. µl/min/g liver	10 mg/kg/b.wt. µl/min/g liver
Rats	1st day	0.63±0.02	0.59±0.03	0.56±0.02
	5th day	0.66±0.01	0.61±0.01	0.35±0.05*
Guinea pigs	1st day	4.51±0.25	4.30±0.39	3.95±0.09
	5th day	4.60±0.15	4.02±0.13	2.72±0.06*

Table (2) Effect of danofloxacin (Advocin) on some biochemical parameters in rats. Velues are expressed as mean ± S. E. (n=5).

Parameter	Time post dosing	Control	Treated	
			5 mg/kg/ b.wt.	10mg/kg/ b.wt.
ALT (unit/ml)	1st day	42.20±3.11	65.00±4.20*	64.25±6.25*
	5th day	40.01±2.55	63.21±4.52*	67.22±3.99*
AST (unit/ml)	1st day	130.80±7.20	159.39±6.00*	172.25±10.25*
	5th day	128.00±7.00	140.50±6.55	139.00±8.00
Urea (mg%)	1st day	19.49±0.84	24.21±2.20*	28.51±4.45*
	5th day	18.93±2.40	26.22±4.00*	25.22±3.00*
Creatinine (mg%)	1st day	0.46±0.03	0.74±0.05*	0.93±0.03*
	5th day	0.55±0.04	0.81±0.04*	0.82±0.01*
Glucose (mg%)	1st day	163.88±6.72	148.00±4.47	166.20±18.41
	5th day	172.35±3.25	164.83±9.41	159.60±20.67
Calcium (mg%)	1st day	8.75±0.63	8.04±0.64	8.00±0.49
	5th day	9.21±0.80	8.52±0.72	9.07±0.32

* Sipnificantly different compared to the control ($P<0.05$).

3. Haematological studies:

Danofloxacin at a dose of 5mg/kg b. wt. i. m. for 5 days singificantly ($P<0.05$) decreased PCV% and RBCs count after 1st day post dosing . Meanwhile, danofloxacin at a dose of 10 mg/kg b. wt. i. m. significantly decreased Hb%, PCV% and RBCs count after 1st day post dosing (Table 3).

4. Effect of danofloxacin on isolated preparations:

Danofloxacin at dose of 40-100 µg/ml failed to induce any effect on the motility of the isoalted rabbit duodenum of guinea pig ileum (Fig. 1 A&B). In addition, it failed to evoke the contractile effect of acetylcholine (2 µg/ml) on the isolated guinea pig ileum and rabbit duodenum.

Table (3): Effect of danofloxacin (Advocin) on some haematological parameters in rats. Velues are expressed as mean ± S. E. (n=5).

Parameter	Time post dosing	Control	Treated	
			5 mg/kg/ b.wt.	10mg/kg/ b.wt.
Hb %	1st day	65.22±4.50	63.80±3.33	42.32±5.21*
	5th day	68.53±7.21	70.25±4.50	62.91±3.29
PCV%	1st day	39.20±1.74	25.39±1.21*	26.25±3.28*
	5th day	38.80±2.05	35.12±1.17	32.29±6.29
RBCs X $10^6/mm^3$	1st day	5.82±0.20	4.62±0.25*	4.15±0.15*
	5th day	5.69±0.15	4.42±0.17	5.52±0.20
WBCs X $10^3/mm^3$	1st day	8.62±0.20	8.43±1.31	8.69±0.12
	5th day	8.80±0.16	8.90±1.00	8.77±0.19

* Sipnificantly different compared to the control ($P<0.05$).

Table (4) Effect of danofloxacin (Advocin) on the frequency of mitosis in bone marrow cells of rats.

Group	No. of examined cells	No. of dividing cells	No. of nondividing cells	Chi-square value
Control	500	42 (8.4%)	458	---
5 mg/kg b. wt.	500	33 (6.6%)	467	0.92
10 mg/kg b. wt.	500	16 (3.2%)	484	11.44**

* Highly singificantly different compared to the control ($P<0.01$).

5. Chromosomal changes:

The effect of danofloxacin on rate of mitosis of bone marrow cells of rats are presented in table (4) and Figs. (2 and 3). The use of danofloxacin at a dose of 5 mg/kg b. wt. had no significant effect on the rate of mitosis (6.6% as compared to that of the control group, 8.4%). On the other hand, it induced a highly significant (P<0.01) decrease in mitotic division (3.2%) at a dose level of 10 mg/kg b.wt. . With respect to chromosomal aberrations, danofloxacin has been found to induce a highly significant effect at 5 and 10 mg/kg b. wt. on the rate of aberrations. The percentages of aberrent cells were 18.2 and 26.6 for the lower and higher doses respectively (Table 5). The results revealed that the drug caused a

Table (5) Effect of danofloxacin (Advocin) on the rate of chromosomal aberrations in bone marrow cells of rats.

Group	No. of examined cells	No. of normal cells	No. of aberrent cells	Chi-square value
Control	500	480	20	---
5 mg/kg b. wt.	500	409	91(18.2%)	53.17**
10 mg/kg b. wt.	500	367	133 (26.6%)	112.50**

* Highly singificantly different compared to the control (P<0.01).

Table (6) Different types of chromosomal aberrations induced by danofloxacin (Advocin) in bone marrow cells of rats.

Group	Total No. of examined cells	F	D	R	G	B	S
Control	500	--	5	--	5	--	10
5 mg/kg b. wt.	500	13	15	5	20	10	28
Chi square value		10.9*	4.13*	3.22	8.04*	8.18**	7.91*
10 mg/kg b. wt.	500	22	20	12	26	20	33
Chi square value		28.25**	8.04*	7..22*	13.32**	18.42**	11.76**

* Significantly different compared to the control (P<0.05)
** Highly significantly different compared to the control (P<0.01)
F= fragment R= ring chromosome D= Chromatid deletion
G= chromatid gap B= chromatid breaks S= stickiness.

dose dependent increase in the percentage of chromosomal aberrations. The different types of chromosomal aberrations are presented in Table (6) and Figs. (5,6 and 7). Chi-square analysis (Table, 6) revelaed that, there was a highly significant effect of the drug on the rate of fragments and breaks at both the dose levels. The lower dose produced only a significant ($P<0.05$) effect on the rate of chromatid deletions, gaps and stickiness and non significant effect on ring chromosome. On the other hand, the dose rate of 10 mg/kg b. wt. induced a higly significant effect on the percentage of gap and stickiness and only a significant effect on the rate of chromatid deletion and ring chromosome.

DISCUSSION

It is evident from the present study that danofloxacin in a dose of 10 mg/kg b. wt. intramuscularly induced a significant decrease in bile flow of rats and guinea pigs. A decline in biliary secretion could be attributed to impairment of the intrahepatic blood flow (Rene et al., 1983). The effect of danofloxacin on intrahepatic hemodynamics is unknown. Danofloxacin inhibits the enzyme DNA gyrase which appeares to essential for DNA replication and subsequently manufacturing of cellular proteins (Wolfson and Hooper, 1985). Protein synthesis inhibitors prevent the stimulation of bile secretion (Simon et

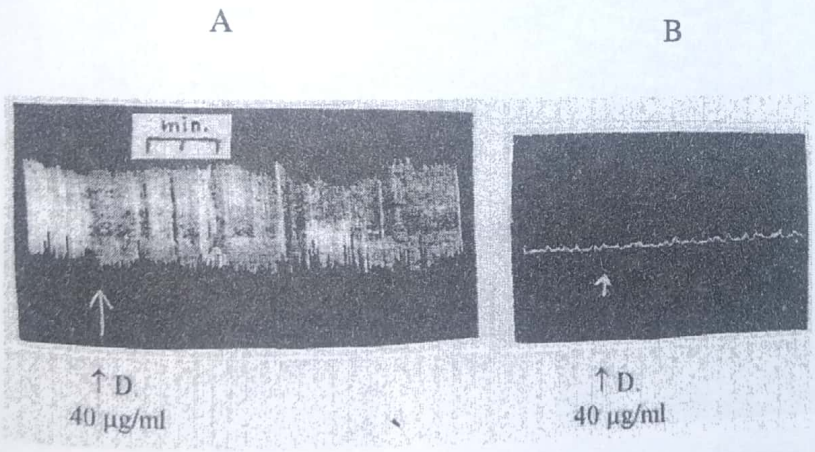

Fig. 1: Effect of danofloxacin (D) on the isolated rabbit duodenum (A) and guinea pig ileum (B).

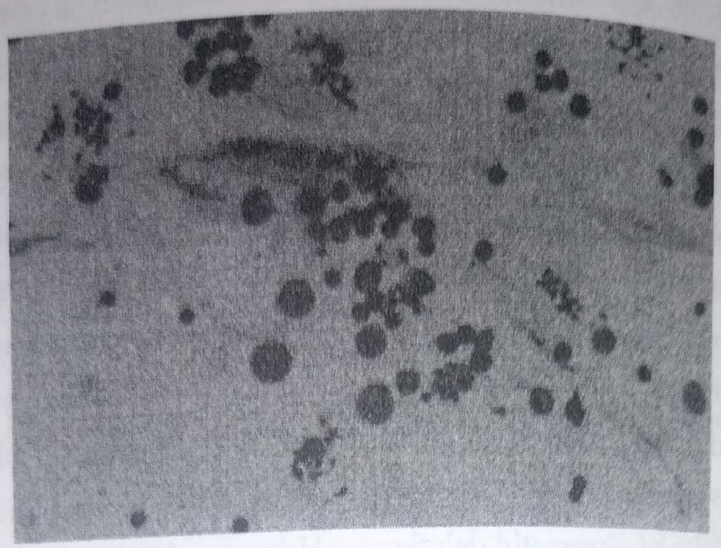

Fig. 2: Normal mitotic activity of control untreated rats.

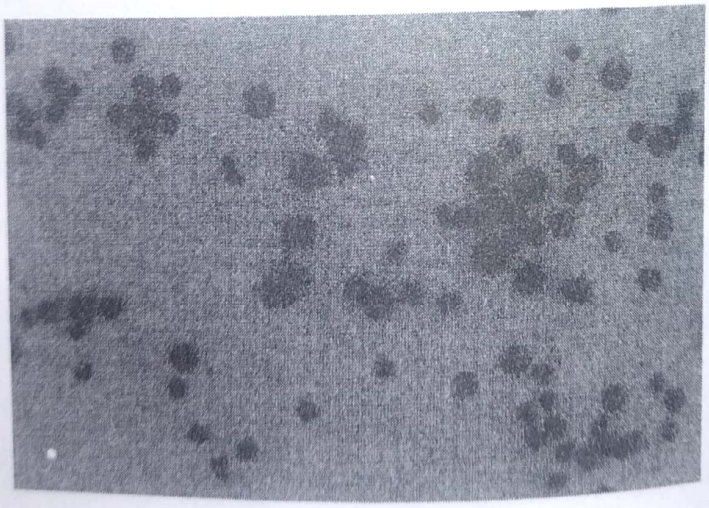

Fig. 3: Inbibition of mitotic activity in rats treated with danofloxacin (10 mg/kg b. w.)

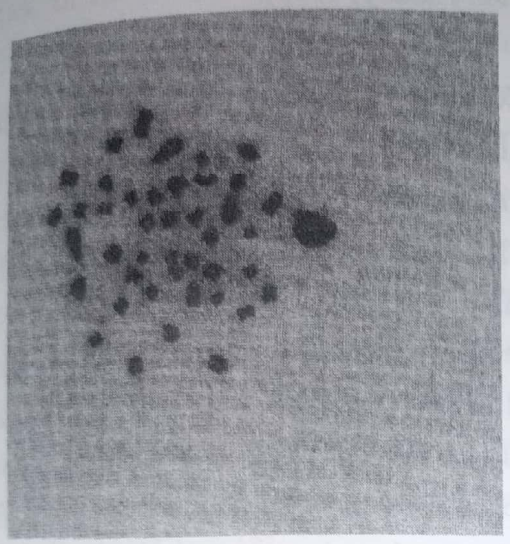

Fig. 4: Normal metaphase chromosomes of bone marrow cells of rats (control group).

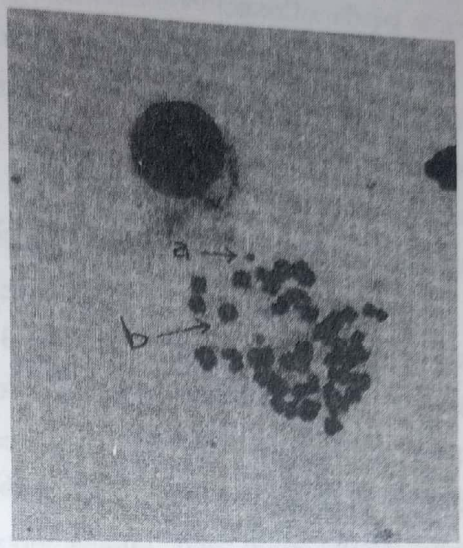

Fig. 5: Metaphase chromosomes of bone marrow cells of rats treated with danofloxacin showing a chromosomal fragments b. ring chromosome.

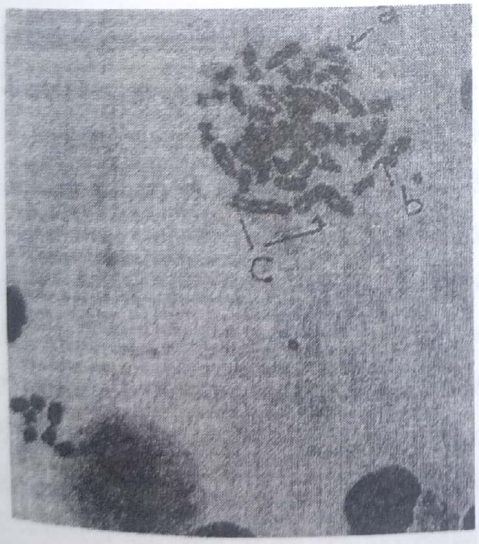

Fig. 6: Metaphase chromosomes of bone marrow cells of rats treated with danofloxacin showing a chromatid deletion.
b. chromatid breaks.
c. chromatid gaps.

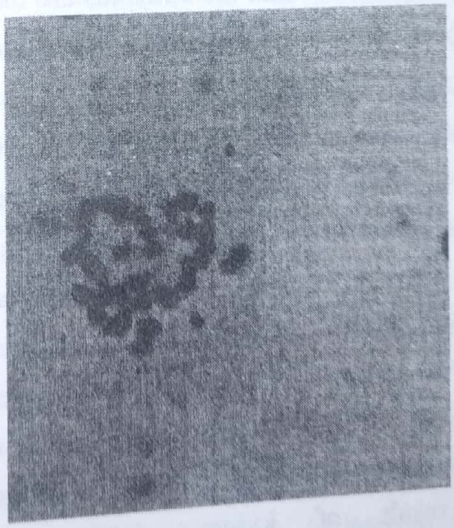

Fig. 7: Metaphase chromosomes of bone marrow cells of rats treated with danofloxacin showing chromosomal stickiness.

al., 1979). Since the liver appears to be the primary site of danofloxacin metabolism (Christ et al., 1988), it might possible that protein synthesis could be impaired and subsequently reduced the bile secretion.

The biliary secretion is controlled to some extent by the stimulation of muscarinic receptors (Kaminski et al., 1974; Rene et al., 1983 and Garwacki et al., (1988). The muscarinic blocking effect of danofloxacin could be excluded as indicated from this study on the isolated intestinal preparations from rabbit and guinea pigs. In addition, it is not clear whether the cholestatic action of danofloxacin is due to the increase in the rate of sodium chloride and water reabsorption from the bile ductule or gall bladder (rat has no gall bladder), specially it is excreted through the bile (Kashida and Kato, 1997), or inhibiting secretin secretion which needs further study.

It is well known that the enzymes are inracellular, being located in mitochondria, the cytoplasm, or both. Consequently, circulating levels increase only following liver damage (Doxey, 1971). The present study revealed that there was a significant increase in serum AST and ALT . Accordingly, this finding might be attributed to damage of the hepatic cells by the direct effect of the drug resulting in escape of these enzymes to the plasma. Furthermore, urea and creatinine significantly increased in serum of trearted rats as a result of kidney damage. Similarly, administration of norfloxacin and other quinolones resulted in elevation of AST, ALT, urea and creatinine (Duffel, 1975; Hillel, 1988 and Hanafy, 1993).

Results of the present study revelaed that danofloxacin has a genotoxic effect. The rate of mitotic division was singifncantly decreased as the dose of the drug increased (Table, 4). Danofloxacin inhibits the enzyme DNA gyrase and so DNA replication (Wolfson and Hooper, 1985). The DNA replication proceeds the cell division and the cell may continue in non dividing phase in the presence of cytotoxic material or abnormal environmental condition. Moreover, the drug was capable of interacting with chromosomes since it was shown to be a positive inducer for many types of chromosomal aberrations (Table 6 and Figs 5 to 7). The structural chromosomal aberrations were summarized as fragments, deletion, rings, gaps and breaks indicate that the danofloxacin has a direct effect on DNA . Some authors (Gebhart, 1977 and Anderson and Richardson, 1981) considered gaps to be a sensitive indicator of chemically induced chromosomal aberrations. Fragments and breaks highly significantly increased at both dose levels and were dose dependent Nichols (1972) correlated chromosomal breaks to gene mutation. On the other hand, the significant induction of chromosomal stickiness indicates that the drug interacts also with chromosomal proteins. Such a conclusion is in agreement with that reported by Mazia (1955 and 1961).

REFERENCES

Anderson, D. and M. S. M. Richardson (1981): Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. Mutat Res., 90(3): 261-272.

Brusick, O. (1988): Principles of genetic toxicology. Plenum Press No. 4, P. 33.

Christ, W.; Lehnert, T. and Ulbrich, B. (1988): Specific toxicologic aspects of the quinolones. Reviews of infectious diseases. Vol. 10 (Suppl. 1) 5141-5146.

Crosby, W. H.; Munn, J. I. and Fruth, F. W. (1954): Standardizing a method for clincial hemoglobinmetry U. S. Arme Force Med. J., 5: 693.

Doxey. D. L. (1971): Veterinary Clinical Pathology. 1st Ed. London, P. 556-560.

Duffel, S. J. (1975): Unexpected reaction to nalidixic acid. Vet. Rec., 96: 188.

El-Ashmawy, I. M.; Fayed, A. H.; Mohamed, M. M. and Abdel Azeez, A. (1994): Effect of neorfloxacin on bile flow in guinea pigs. Vet. Med. J., Giza, 42 (2): 9-14.

Epi-Info (1994): "Version 6,A word-processing, database and statistics program for public health on IBM-compatible microcomputers. "Designed by Dean, A. G.; Dean, J. A.; Coulombier, D.; burton, H. A.; Brendel, K. A.; Smith, D. C., Dicker, R. C.; Sullvian, K. M.; and Fagan, R. C. and produced by the Division of Surveillance and Epidemiology, Epidemiology program Office, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 30333 in collaboration with The Global Programme on AIDS, World Health Organization (WHO), Geneva, Switzerland.

Garwacki, S.; Dabrowski, J. and Dutkiewicz, M. (1988): Effect of carbachol and atropine on bile production in sheep. Polskie Achiwum Weterynaryine, 28 (3-4): 71-77.

Gebhart, E. (1977): Experimentelle Beitrage zum problem der Lokalen Achromasien (Gaps). Humangenetik, 13: 98-107.

Hanafy, A. M. (1993): Some adverse effects of norfloxacin in male rats. Thesis presented to Fac. Vet. Med. Alex. Univ. for the degree of M. V. Sc. (Pharmacology).

Hemeda, Sh. A. (1994): Effect of chloramphenicol on mitosis and chromosomes in albino rats. Egypt. J. Appl. Sci., 9 (12): 875-991.

Hillel, H. (1988): Adverse effects of fluorquinolones. Rev. Infect. Dix., 10 (Suppl. 1): 258-261.

Hoyt, D. and Larson, R. E. (1989): Cholestatic effect of carmustine in rats. J. Pharmacol. and Exp. Therap, 249 (1): 165-171.

Jackson, S. (1975): Creatinine method developed by Jackson, S., Hospital for sick children. Toronto and presented at the International Congress of Clinical Chemistry, Toronto, July, 1975.

Kaminski, D. L.; Dorighi, J. and Jellinex, M. (1974): Effect of electrical vagal stimulation on canine hepatice bile flow. Am. J. Physiol, 227 (5) 487-498.

Kashida, Y. and Kato, M. (1997): Characterization of fluoroquinolone induced achilles tendon toxicity in rats: comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. Antimicrob. Agents Chemother., 41(11): 2389-2393.

Macgregor, H. C. and Varely, J. M. (1983): Working with animal chromosomes. Chapter 11; P. 32. A Wiley-Interscience Publication, John Wiley and Sons, Chickester, New York, Toronto Singapore.

Magnus (1904): Cited in pharmacological experiments on isolated preparations by the Staff of Pharmacology Dept. Univ. of Edinburgh (1970). Livingstone, E & S Edinburgh, London, 2nd Ed. P. 62.

Maha, A. Fahmy and M. S. M. Hanafy (1992): Rifampicin genotoxicity in mice Proc. 2nd Cong. Fac. Vet. Med., Cairo Univ.

Mann, D. D. (1989): Efficacy of quinolone antibiotic danofloxacin in food producing animal disease models, 29th Interscience Conference of Antimicrobial Agents and Chemotherapy, Houston, Texas, Abstract 1189.

Masuda, N.; Takahashi, Y.; Otsuki, M.; Ibuki, E.; Miyoshi, H. and Nishineo, T. (1996): In vitro and in vivo antibacterial activities of CS-940, a new 6-fluoro8-difluoromethoxy quinolone. Antimicrob. Agents Chemother, 40 (5). 1201-1207.

Mazia, D. (1955): The organization of the mitotic apparatus. Sym. Soc. Exp. Biol, 9: 335-357.

Mazia, D. (1961): Mitosis and physiology of cell division. In the cell. J. Brachet and Mirsky, A. E., eds, 3: 77-412.

Mnlazimoglu, L.; Drenning, S. D. and Yn, V. L. (1996): In vitro activities of two novel oxazolidinones (U100592 and U100766), a new fluoroquinolone (Trovafloxacin) and dalfopristin-quinupristin against *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother, 40(10): 2428-2430.

Nichols, W. W. (1972): Genetic hazards of drugs of abuse. In Crafonetics, C. J. D. (ed) "Drug abuse". Proceedings of International Conference, Lea and Febiger.

Paget, G. E. and Barnes, J. M. (1964): Toxicity tests, Chapt, 6. P. 135-166. In Evaluation of drug activities pharmacometrics. Vol. 1. Edited by Laurance. D. R. and Bacharach, A. L., Academic Press, London and New York.

Patton, C. J. and Crough, S. R. (1977): Colorimetric determination of urea. Anal. Chem, 49: 464-469.

Reitman, S. and Frankel, S. (1957): A colorimetric method for determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Path, 28: 56-63.

Rene, E.; Danzinger, R. G.; Hofmann, A. F. and Nokagaki, G. M. (1983): Pharmacologic effect of somatostatin on bile formation in the dog Gastronenterology, 48 : 120-129

Schalm, O. W. (1975): Veterinary haematology, 2nd Ed Lee and Febiger, Philadelphia.

Siest, G.; Henny, J. and Schiele, F. (1981): Interpretation des examens de laboratori, Krager, ed. 206-223.

Simon, F. R.; Johnson, W. P.; Miner, P. and Sutherland, E. (1979): Effect of phenobarbital administraiton on hepatic oxygen consumption and the apparent induction of Na/K ATPase in the liver. J. Clin. Invest, 50: 849-861.

Snedecor, G. W. and Cochran, W. G. (1980): Statistical methods. 6th Ed. Iowa State Univ. Press, Ames, USA.

Tanner, A. C.; Avakian, A. P.; Barnes, H. J.; Ley, D. H.; Miagaki, T. T. and Magonigle, R. A. (1993): A comparison of danofloxacin and tylosin in the control of induced *Mycoplasma gallisepticum* infection in broiler chicks. Avian Dis, 37: 515-522.

Tietz, N. W. (1970): Fundamentals of Clinical Chemistry, W. B. Saunders, Philadelphia.

Wolfson, J. S. and Hooper, D. C., (1985): The fluoroquinolones: structures, Agents Chemother., 28: 581-586.