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Teratogenic effects of ciprofloxacin and pefloxacin in pregnant rats

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SUMMARY

The adverse effects of ciprofloxacin and pefloxacin on rat fetuses following oral administration to the pregnant rats were studied. Ciprofloxacin and pefloxacin were given daily to pregnant rats at two (therapeutic and double therapeutic) dosage levels during the period of organogenesis (from the 6th to the 15th day of gestation). Oral administration of ciprofloxacin at 4.5 and 9 mg/100 g.b.wt.to pregnant rats induced fetal resorption, death and growth retardation. Visceral examination of live fetuses showed microcephaly, hypoplasia of the heart and lungs and dilatation of renal pelvis. Skeletal examination revealed incomplete ossification of skull bones and absence of some coccygeal vertebrae. Pefloxacin at 7.2 6 mg/100 g. b. wt. decreased the number of live fetuses and caused microcephaly, hypoplasia of the heart and lungs, dilatation of the renal pelvis. It also induced incomplete ossification of skull bones and absence of sternbrae and phalanges of forelimbs. A great attention should be considered during the clinical use of ciprofloxacin and pefloxacin in pregnant women and animals to avoid their teratogenic effects.

Key words: Ciprofloxacin; Pefloxacin; Pregnancy; Fetus; Teratogenicity

INTRODUCTION

Ciprofloxacin and pefloxacin are two fluoroquinolone antibacterial drugs which had been commonly used for treating many microbial infections in humans and in veterinary medicine (Prescott *et al.*, 2000). There has been an increasing concern about the female reproductive toxicity and teratogenic risk of fluoroquinolones. These are of wide spread use in human and veterinary medicine and possess properties that allow them to cross the placental barriers and reach the mammalian fetus, so they can cause embryotoxicity and / or teratogenicity (Neu, 1988 and Giamarellou *et al.*, 1989).

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Some members of fluoroquinolones induced embryotoxicity and / or teratogenicity when given to pregnant experimental animals, while others were neither embryotoxic nor teratogenic. Ofloxacin have no evidence of teratogenicity when given orally to pregnant rats and rabbits, but only rat fetuses in the high dose of ofloxacin exhibited skeletal abnormalities represented by retarded ossification (Takayama *et al.* 1986). Tesh *et al.* (1988 A&B) found that lomefloxacin had no effect on the development of the fetuses till a dosage level of 300 mg/kg/day. Cukierski *et al.* (1989) reported no evidence of teratogenicity to norfloxacin in monkeys at any dose level. Funabashi *et al.* (1991) recorded an increased incidence of ventricular septal defect, a decreased incidence of the 14th rib defect, and delayed ossification in the fetuses after administration of sparafloxacin to pregnant rats. Levofloxacin showed teratogenic effects in rats and rabbits only at the high dose levels (Watanabe *et al.* 1992). Oral administration of high doses (300 and 3000 mg/kg.b.wt.) of prulifloxacin to pregnant rats decreased fetal body weight and caused delayed ossification of skeleton bones (Komae *et al.* 1995 and Morinaga *et al.* 1996 B). On the other side, Morinaga *et al.* (1996A) and Komae *et al.* (1998) mentioned that no teratogenic effects were found after administration of ofloxacin, temafoxacin, and enrofloxacin to pregnant rats and rabbits.

Since the teratogenic effects of some fluoroquinolones still need further investigations, so the present study was designed to investigate the adverse effects of ciprofloxacin and pefloxacin on the fetuses after oral administration to pregnant rats during the period of organogenesis.

MATERIALS AND METHODS

1. Drugs:

Ciprofloxacin and pefloxacin antibacterial drugs were obtained as pure powders from Amirya Company for Pharmaceutical Industries, Egypt. Each drug was suspended in 0.5 % of carboxy methylcellulose. The tested doses therapeutic and double therapeutic) were calculated according to the conversion table of Paget and Barnes (1964)

2. Rats:

Adult female Sprague Dawley rats weighing 175-185 g were used in this study. Rats were obtained from Laboratory Animal Colony, Helwan, Egypt, and fed on standard rat pellets. Water was provided *ad libitum* and animals were left for a week before start of the experiment for acclimatization.

3. Experiment:

Female rats were examined periodically using a vaginal smear test to ensure that they were in a regular estrous cycle (Cahen, 1966). Each female in estrous phase was paired with a proven fertile male overnight. Zero day of

pregnancy was determined by presence of spermatozoa in the examined vaginal smear next morning (Barcellona *et al.*, 1977). Pregnant rats were divided into 5 equal groups. The 1st group was kept as a normal control and given 1 ml of vehicle (carboxy methylcellulose). Whereas the 2nd and 3rd groups were given ciprofloxacin at 4.5 and 9 mg/100 g. b. wt., respectively. The 4th and 5th groups were administered pefloxacin at 3.6 and 7.2 mg/100 g.b.wt., respectively. Both drugs were given orally during organogenesis i.e. from the 6th to the 15th days of gestation (Tuchmann-Duplessis, 1975). The control and treated female rats were observed daily for gross appearance and behavior till the 20th day of gestation. Female rats were then euthanized by ether anesthesia. Live and dead or resorbed fetuses were distinguished and counted and live fetuses were subjected to morphological, visceral, and skeletal examinations according to Cook and Fair-weather (1968), Wilson (1965) and Staples and Schnell (1964), respectively.

4. Statistical analysis:

The obtained data were expressed as mean ± S.E. and percentages. Statistical analyses were carried out according to Snedecor and Cochran (1986) using paired Student t- test and Chi- square test for percentages analysis.

RESULTS

There are no any notable changes in behavior or clinical sings were observed the control and treated dams.

Morphological abnormalities of the fetuses obtained from the pregnant rats given orally ciprofloxacin or pefloxacin during the period of organogenesis are recorded in table (1) and demonstrated in Figs. (1-2). Oral administration of ciprofloxacin at 9.0 mg/100 g. b. wt. significantly ($P \leq 0.001$) decreased the percentage of live fetuses to 91.9% compared to the control group. It also induced early fetal resorption (Fig.1) by 3.2 %. Growth retardation (Fig.2) was evident by significant decreases of fetal body weight and length associated with fetal death by 4.9 %. Oral administration of pefloxacin to pregnant rats in a dose of 3.6 mg/100 g. b. wt. showed non significant morphological changes in live fetuses. The large dose (7.2 mg/100g.b.wt.) of pefloxacin caused a significant ($P \leq 0.01$) decrease in the percentage of live fetuses to 95.5 % compared to control group and fetal resorption by 4.5%.

Visceral malformations of the fetuses obtained from the pregnant rats given orally ciprofloxacin and pefloxacin during the period of organogenesis are depicted in table (2) and shown in Figs. (3-4). Ciprofloxacin administration at both tested doses (4.5 and 9.0 mg/100 g b.wt.) caused hypoplasia of the lungs and dilatation of the renal pelvis.

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Microcephaly (Fig. 3) and hypoplasia of the heart and lungs (Fig.4) were only seen by the large dose. Pefloxacin when given orally to the pregnant rats produced hypoplasia of the heart and lungs at both doses (3.6 and 7.2 mg/100 g b.wt.), while microcephaly and dilatation of the renal pelvis were only seen by the large dose.

Skeletal malformations of the fetuses obtained from the pregnant rats given orally ciprofloxacin and pefloxacin during period of organogenesis are recorded in Table (3) and illustrated Figs. (5-7). Ciprofloxacin at both doses (4.5 and 9 mg/100 g b.wt.) induced incomplete ossification of skull bones (Fig.5). Absence of some coccygeal vertebrae (Fig.6) was only seen with the high dose. Pefloxacin induced incomplete ossification of skull bones and absence of sternbrae at both tested doses (3.6 and 7.2 mg/100 g b.wt.), while absence of phalanges of forelimbs (Fig. 7) was only seen by the large dose.

Table (1): Fetal morphological abnormalities after oral administration of ciprofloxacin and pefloxacin to pregnant rats during period of organogenesis (mean ± SEM).

Groups	Doses (mg/100 g. b. wt.)	No. of live fetuses/ dam		No. of dead fetuses/dam		No. of resorbed fetuses/dam		Fetal b. wt. (g) Mean± SE	Fetal length (mm) Mean± SE
		Mean	%	NO	%	NO	%		
Control	1 ml CMC	7.9 ± 0.2	100	0	0	0	0	3.6 ± 0.08	38.4 ± 0.5
Ciprofloxacin	4.5	7.3± 0.3	100	0	0	0	0	2.7± 0.01**	37.8± 0.2
	9.0	5.3 ± 0.2***	91.9**	2	3.2	3	4.9	2.2±0.09***	34.0±0.6***
Pefloxacin	3.6	7.1 ± 0.3	100	0	0	0	0	3.4± 0.09	37.2 ± 0.6
	7.2	6.8 ± 0.2**	95.5**	0	0	4	4.5	3.5± 0.06	37.8± 0.6

n = 10 pregnant rats
b. Wt. = body weight
** Significant at P ≤ 0.01
*** Significant at P ≤ 0.001
CMC: Carboxymethyl cellulose (vehicle)

Table (2): Visceral malformations of fetuses after oral administration of ciprofloxacin and pefloxacin to pregnant rats during period of organogenesis.

Groups	Doses mg/100 g.b.wt.	No of examined fetuses	Visceral malformations in							
			Brain		Heart		Lung		Renal Pelvis	
			No.	%	No.	%	No.	%	No.	%
Control	1 ml CMC	55	0	0	0	0	0	0	0	0
Ciprofloxacin	4.5	52	0	0	0	0	6	11.5	4	9.7
	9.0	41	2	4.8	2	4.8	8	19.5	18	34.6
Pefloxacin	3.6	50	0	0	6	12.0	12	24.0	0	0
	7.2	46	2	4.3	10	21.7	16	34.7	6	13.0

n = 10 pregnant rats
CMC: Carboxymethyl cellulose (vehicle)

Table (3): Fetal skeletal malformations after administration of ciprofloxacin and pefloxacin to pregnant during period of organogenesis.

Groups	Doses mg/100 g.b.wt	No.	Malformations in									
			Skull		Ribs		Sternbrae		Forelimbs		Coccygeal Vertebrae	
			No	%	No	%	No	%	No	%	No	%
Control	1 ml CMC	24	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin	4.5	21	17.0	77.2	0	0	0	0	0	0	0	0
	9.0	22	18.0	85.7	0	0	0	0	0	0	3	13.6
Pefloxacin	3.6	21	4.0	19.0	0	0	6.0	28.5	0	0	0	0
	7.2	22	6.0	27.2	4.0	18.1	8.0	36.3	3.0	13.6	0	0

n = 10 pregnant rats NO. = number of examined fetuses
CMC: Carboxymethyl cellulose (vehicle)

Morphological examination of rat fetuses showing:

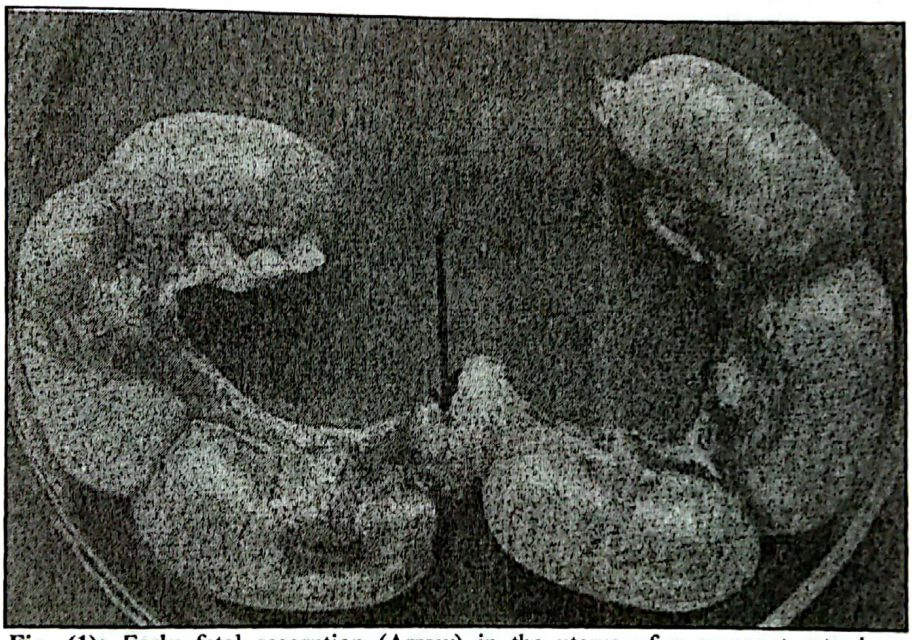

Fig. (1): Early fetal resorption (Arrow) in the uterus of a pregnant rat given ciprofloxacin (9.0 mg/100g.b.wt.) from the 6th to the 15th day of gestation.

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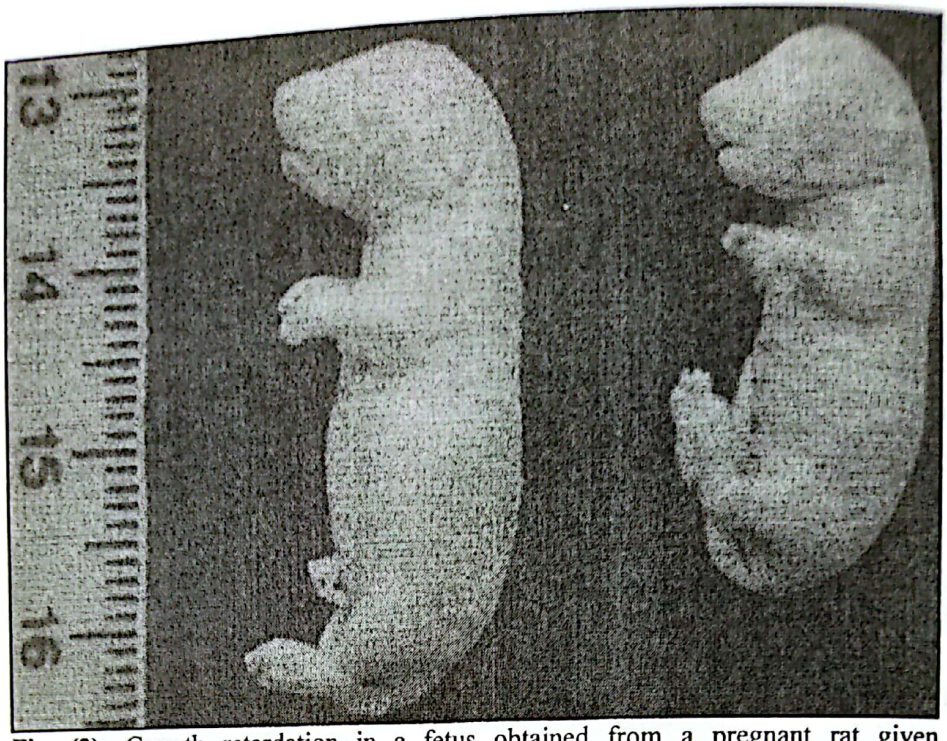

Fig. (2): Growth retardation in a fetus obtained from a pregnant rat given ciprofloxacin (9.0mg/100g.b.wt) from the 6th to the 15th day of gestation. (Left: Control Right: Growth retarded fetus).

Visceral abnormalities of rat fetuses:

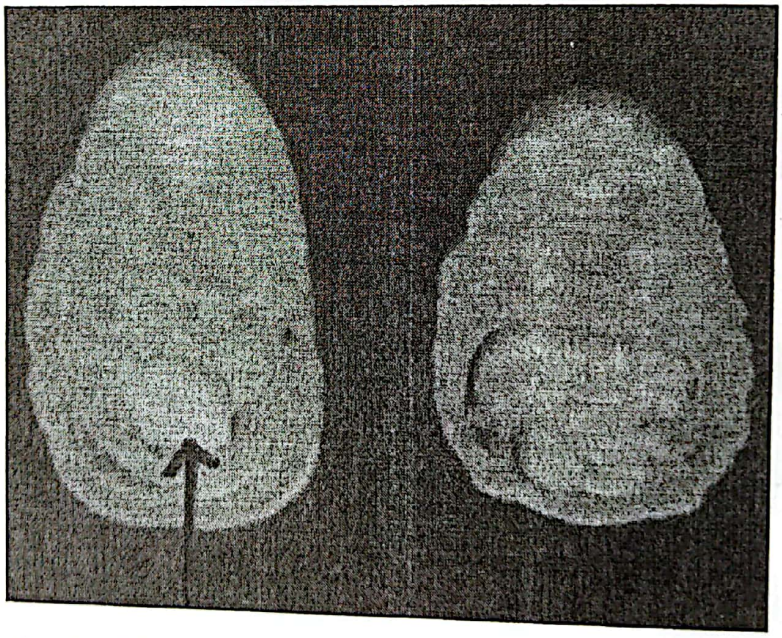

Fig. (3): Microcephaly (Arrow) in a head of fetus obtained from a pregnant rat given pefloxacin (7.2 mg/100g.b.wt).from the 6th to the 15th day of gestation.

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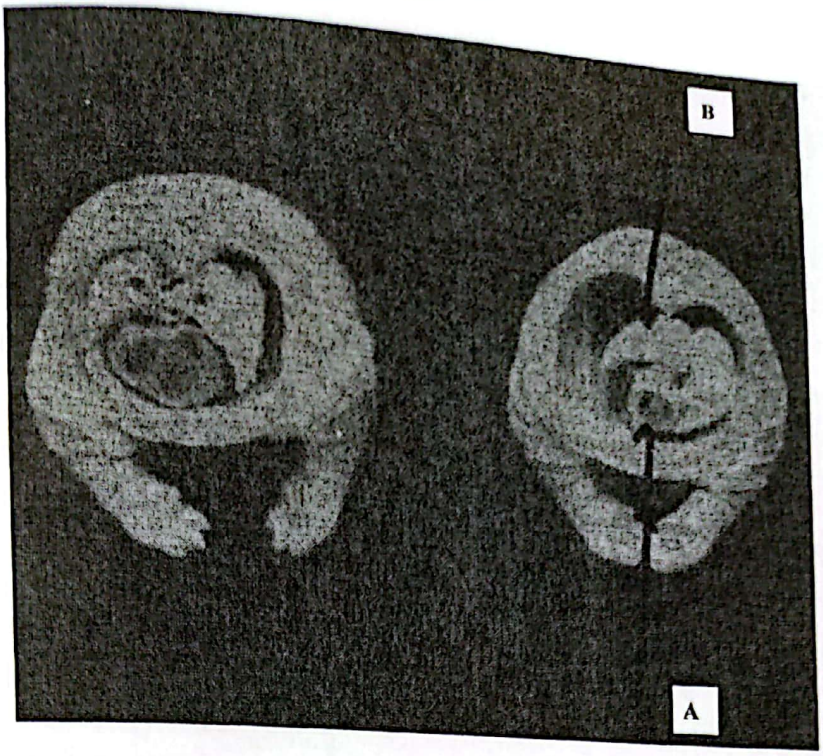

Fig. (4): Hypoplasia of heart (A) and lungs (B) of a fetus obtained from a pregnant rat given ciprofloxacin (9.0mg/100g.b.wt).from the 6th to the 15th day of gestation.

Skeletal abnormalities of rat fetuses:

Fig. (5): Incomplete ossification in the skull bones of a fetus obtained from a pregnant rat given ciprofloxacin (4.5mg/100g.b.wt) from the 6th to the 15th day of gestation.

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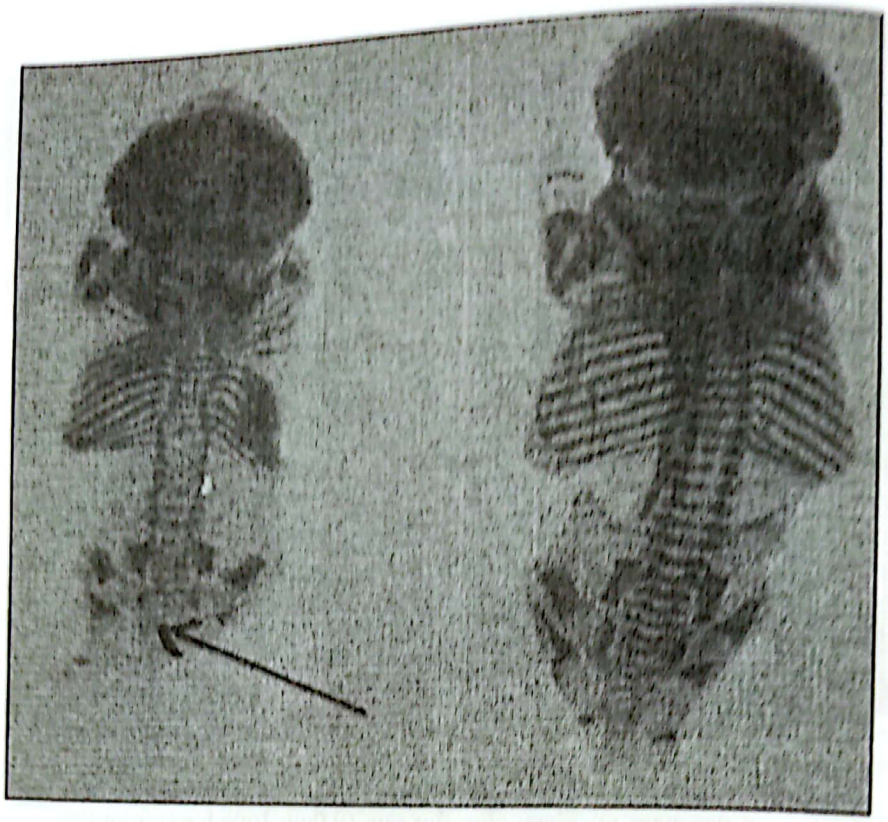

Fig. (6): Absence of coccygeal vertebrae in a fetus obtained from a pregnant rat given ciprofloxacin (9.0mg/100g.b.wt) from the 6th to the15th day of gestation

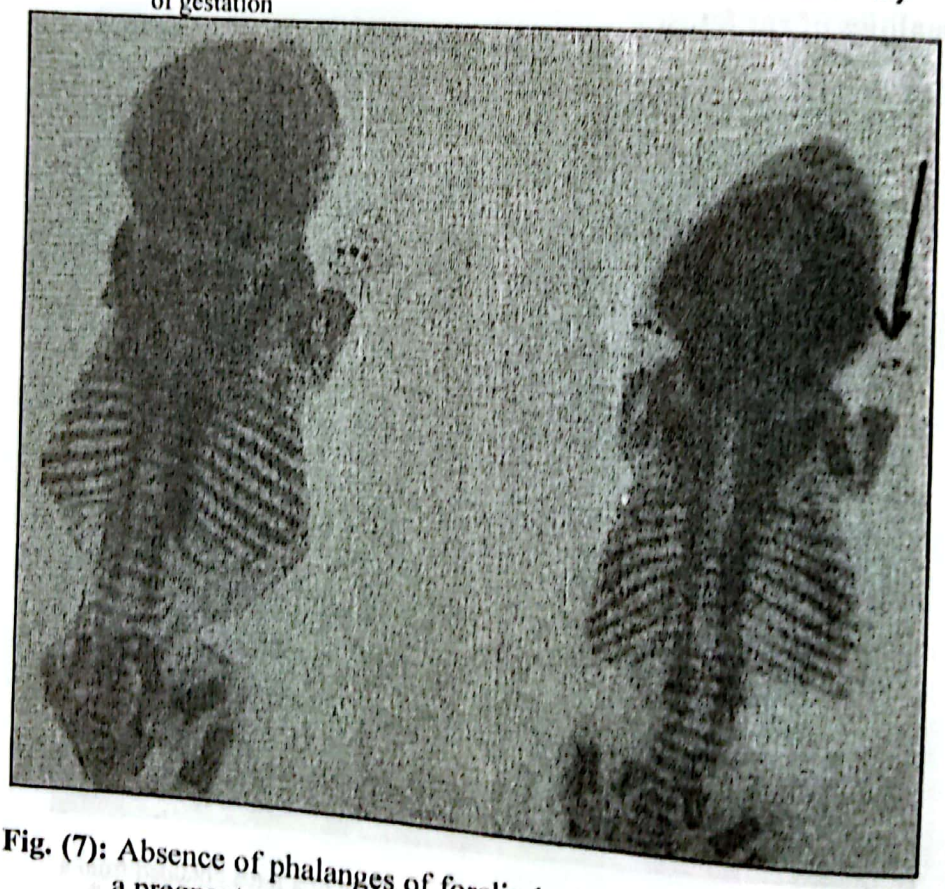

Fig. (7): Absence of phalanges of forelimbs in a fetus obtained from a pregnant rat given pefloxacin (7.2mg/100g.b.wt.) from the 6th to the 15th day of gestation.

(Arrows refer to fetal abnormalities)

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DISCUSSION

In the present work, the teratogenic effects of two commonly used fluoroquinolones namely ciprofloxacin and pefloxacin were investigated in pregnant rats. The obtained results are similar to those recorded by Takayama *et al.* (1986) who found that oral administration of fluoroquinolones to pregnant rats and rabbits during the period of organogenesis caused delayed ossification and skeletal malformations in the fetuses. Moreover, oral administration of ofloxacin to pregnant rats at high doses induced skeletal abnormalities in their foetuses. Althreuther (1987) also recorded maternal toxicity and embryonic deaths in rats, mice, and rabbits after fluoroquinolones administration.

Similar findings were reported by Funabashi *et al.* (1991), Watanabe *et al.* (1992), Komae *et al.* (1995) and Morinaga *et al.* (1996B). The authors found that fluoroquinolones (sarafloxacin, levofloxacin, T-3761, a new quinolone derivative, and prulifloxacin) when given to pregnant rats induced ventricular septal defect, delayed ossification, decreased body weight, and increased mortality rate.

On the other hand, our results disagree with those reported by Schulter (1989) who mentioned that oral and parental administrations of ciprofloxacin to monkeys had no maternal or embryonic toxic effects. This disagreement may be due to the differences in the species, doses, and route of administration. Also, Tesh *et al.* (1988A&B), Davis and McKenzie (1989), Cukierski *et al.* (1989), Barragry (1994), Berkovitch *et al.* (1994), Morinaga *et al.* (1996A&C), and Loebstein *et al.* (1998) did not find any adverse effects of some fluoroquinolones (ofloxacon, norfloxacin, temafoxacin and prulifloxacin) on the development of fetuses in experimental animals.

The embryotoxic effects of ciprofloxacin and pefloxacin reported in the present study could be attributed to their direct cytotoxic action on the fetus as they can cross the placenta (Neu, 1988 and Giamarellou *et al.*, 1989). The teratogenic effect of fluoroquinolones may be explained also by their inhibition of topoisomerase and the binding of quinolones to topoisomerase DNA complex results in damage to DNA and induction of chromosomal aberration (Takayama *et al.*, 1995). Delayed ossification and skeletal malformations in the fetuses following fluoroquinolones administration could be due to deficiency in maternal nutritional supply because pregnant rats receiving a higher dose of fluoroquinolones exhibited soft stool and enlargement of cecum resulting from imbalance of intestinal bacterial flora (Takayama *et al.*, 1986).

In conclusion, both ciprofloxacin and pefloxacin when given to pregnant rats during the period of organogenesis can adversely

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affect fetal development causing some morphological, visceral, and skeletal abnormalities. Therefore, a great attention should be taken when these drugs are used during pregnancy to avoid the teratogenic and embryotoxic effects.

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التأثيرات المشوهة للأجنة لدوائى سبروفلوكساسين وبفلوكساسين فى الفئران الحوامل

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استهدف البحث دراسة التأثيرات الضارة لمضادى البكتريا سبروفلوكساسين وبفلوكساسين (من مجموعة الفلوروكينولات) على أجنة الفئران بعد إعطائهما عن طريق الفم بجرعتين(العلاجية وضعف العلاجية) لكل منهما. تم اعطاء السبروفلوكساسين لإناث الفئران بجرعة ٤,٥ و ٩,٠ مجم / ١٠٠ جم من وزن الجسم والبفلوكساسين بجرعة ٣,٦ و ٧,٢ مجم / ١٠٠ جم من وزن الجسم وذلك طوال فترة تكوين الأعضاء الداخلية للأجنة (من اليوم السادس إلى اليوم الخامس عشر من فترة الحمل). وتمت التضحية بالفئران الحوامل فى اليوم العشرين من الحمل لفحص الأجنة الموجودة فى أرحامها.

أوضحت النتائج أن اعطاء السبروفلوكساسين والبفلوكساسين عن طريق الفم بجرعتين لكل منهما للفئران الحوامل قد أحدث تأثيرات سمية ومشوهة للأجنة. فقد أدى السبروفلوكساسين إلى حدوث إمتصاص مبكر وموت لبعض الأجنة، ونقص فى عدد الأجنة الحية وأوزنها و أطوالها. ولوحظ أيضا وجود ضمور فى الرئتين و القلب، إتساع فى الحوض الكلوى و صغر حجم المخ فى الأجنة . و كشف فحص الهيكل العظمى للأجنة عن حدوث تمعظم غير كامل لعظام الجمجمة وغياب بعض الفقرات العصعصية. وأدى إعطاء البفلوكساسين للفئران الحوامل إلى حدوث نقص فى عدد الأجنة الحية، ضمور فى القلب و الرئتين ، صغر حجم المخ و إتساع الحوض الكلوى. أما تشوهات الهيكل العظمى فكانت فى شكل تمعظم غير كامل لعظام الجمجمة و غياب لعظام القص و سلاميات الأطراف الأمامية. وتوصى الدراسة بتوخى الحذر والحيطة عند الإستخدام الإكلينيكى للسبروفلوكساسين والبفلوكساسين فى علاج العدوى البكتيرية أثناء فترة الحمل فى الانسان والحيوانات وذلك لتجنب تأثيراتهما السمية والمشوهة للأجنة.