EMBRYOTOXICITY OF FLUOROQUINOLONES IN RATS

AL-Myahi A. J. * , AL-Musawy A. A. H. *, Al-Snafi, Ali Esmail**,

ABSTRACT

This study was designed to investigate the possible developmental fetotoxicity and teratogenicity of Norfloxacin (NFX), Ciprofloxacin (CPX) and Enrofloxacin (ENX) in rats. Eighty pregnant female rats were divided into four equal groups, the first three groups were given daily single oral doses of NFX,700mg/kg/day, CPX 550mg/kg/day, and ENX 750mg/kg/ day respectively and the last group was given dimethyl sulphoxide (DMS) 0.5 ml/animal/day. All animals administered the drugs and the vehicle from the day 1 to the day 15 of gestation period . Ten animals from each group were sacrificed at day 15 of gestation by cervical dislocation, the other ten animal from each group were left till labor. Embryos and pups were evaluated for litter size ,weight, resorption ratio, fetal lost and external features . The effect of drugs on the length of gestation period was also evaluated . The results revealed that fluroquinolones were significantly decrease litter size, and fetal weight, and increase fetal resorption ratio and fetal lost as compared to control either fetuses evaluated at day 15 of pregnancy or those evaluated after birth . Gross examination showed some teratogenic abnormalities in fetuses and pups of mothers treated with fluoroquinolones during the first 15 days of pregnancy .

INTRODUCTION

During the past few decades, it has become increasingly evident that human and animal embryos are subjected to the toxic effects of many drugs (Amwayi and Otiang, 1997) , such as the use of some antibiotics in the treatment serious diseases occurring during pregnancy. Fluoroquinolones are one of the main classes of antimicrobials used in the treatment of many infections . Norfloxacin (NFX) (1- ethyl -6- fluoro -1,4- dihydro -4- oxo -7- 1piperazinyl)-3-quinoline carboxylic acid), is a synthetic, broad spectrum antibacterial fluoroquinolone for oral administration. It has activity against the common pathogenic gram-negative organisms that cause urinary tract infections, including Enterobacter, Pseudomonas aeroginosa, and Neisseria spp. (Katzung, &Trevor, 2008). Ciprofloxacin (CPX) (1-Cyclopropyl -6- Fluoro-1, 4- Dihydro -4- Oxo -7- (1- Piperazinyl) -3- Quinolonecarboxylic Acid) is one of the second generation fluroquinolones with an extended spectrum (McKellar et al., 1999). It has a great activity against gram-negative bacteria and gonococcus, many gram-positive cocci, mycobacteria, and agenst of atypical pneumonia such as Mycoplasma pneumonia, and Chlamydophila pneumonia are also sensitive to it (Katzung ,& Trevor, 2008). Enrofloxacin (ENX) (1-cyclopropyl -7- (4- ethyl -1-

* Department of Physiology, College of Vet. Medicine, Basrah University.
** Department of Pharmacology, College of Medicine, Thi-Qar University.

piperazinyl) -6- fluoro -1 , 4 - dihydro – 4 - oxo -3 quinolonecarboxylic acid) is a synthetic, broad spectrum fluroquinolone (Wolfson, & Hooper, 1989). It was used exclusively in veterinary medicine for the treatment of septicemia, respiratory tract, urinary tract, skin, soft tissues, bone and joint infections (Sanjib et al., 2005). Because fluroquinolones are mainly used now for the treatment of urinary tract infections which have high incidence during pregnancy especially in the first trimester, and because fluroquinolones exert their effects via inhibition of nucleic acid synthesis, therefore this study was designed to evaluate the safety of these drugs on embryonic development.

MATERIALS & METHODS

Eighty adult virgin Norwegian white females (Rattus norvigicus), 10-12 weeks old , weighing (175-250) grams were obtained from the animal house in the collage of education, Thi-qar university . Animals were maintained under special conditions: temperature (25±4) C^{o} and 14:10 light/dark cycle. Water and food were available ad libitum (Agrawala et al, 1968). After 2 weeks of acclimatization, the fertility of females was checked with daily vaginal smear examination for 4 pre-treatment estrous cycles (Marcondes et al., 2002) to establish their normal pattern of cyclical activity. The females during proestrus stage were caged overnight with fertile normal healthy males (2 males and 4 females in each cage) of the same strain . The presence of sperms in the vaginal smear was considered as day 1 of pregnancy. Pregnant females were divided into 4 groups (20 pregnant females for each group) . They were given Norfloxacin 700mg.kg–1/day , Ciprofloxacin 550 mg.kg–1/day , Enrofloxacin 750 mg.kg–1/day , and 0.5 mL of DMS (dimethyle sulphoxide , the vehicle to serve as control) respectively . All treatments were given orally as a single daily dose from 1st to 15th day of gestation. Half number from each group were killed at the day 15 of gestation, while the other half left to labor. Fetuses and neonates were observed for number, weights, fetal resorptions ratio , fetal lost, and teratogenic features.

RESULTS

-The effects of fluoroquinolones in females killed at the 15 of gestation

The effects of fluoroquinolones on litter size:

The effects of single daily doses of Norfloxacin 700 mg/kg/day, Ciprofloxacin 550 mg/kg/day, and Enrofloxacin 750 mg/kg/day administered orally from the first to the fifteenth day of gestation on the litter size was summarized in table (1). The mean of litter size in Norfloxacin , Ciprofloxacin and Enrofloxacine treated groups was (6.400±2.302) , (6.111±3.620) and (6.555±2.006) respectively. The litter size in all treated groups was significantly($P< 0.01$) decrease in comparison with control group . However these results indicate that fluoroquinolones have high dominant mutation index (DLMI) according to the equation of Bader and Bader (1975).

$$DLMI = 1 - \frac{\text{Litter size in treated group}}{\text{Litter size in control group}} \times 100$$

The effects of fluoroquinolones on fetal weight:

The effects of NFX, CPX, & ENX on the fetal weight of treated females was showed in table (2). The mean of fetal weights of pregnant female rats treated with Norfloxacin ,Ciprofloxacin and Enrofloxacin was (0.977±0.179 gm), (0.823 ± 0.405 gm.) and (0.823 ± 0.405) respectively . Fetal weights in all treated

groups were significantly (P< 0.01) less than weights of fetuses in control group). The sections of embryos at the day 15 of gestation showed delay in the processes of chondrogenesis and ossification processes in vertebrae as compared to embryos in control group, which showed a larger areas of ossification (figure1).

The effects of fluoroquinolones on fetal resorption rate:

The mean of resorped fetuses in the groups treated with Norfloxacin, Ciprofloxacin and Enrofloxacin was was (2.200 ± 0.836), (3.000 ± 2.828), and (3.428 ± 1.988) respectively , in comparison with the control group (0.20 ± 0.632) (table 3). The mean of resorped fetuses was significantly increased in all treated groups compared with control.

Fetal lost caused by fluoroquinolones :

The mean of fetal lost in treated animals was shown in table (4). It appeared that Ciprofloxacin was significantly (P<0.05) increase fetal lost in comparison with the control group. However this effect was more prominent in Norfloxacin and Enrofloxacin treated groups (P< 0.01) .

The effects of fluoroquinolones on external features:

The mean of external malformations occurred in treated animals was summarized in table (5). No external malformations were recorded in control group. But they were recorded in all fluroquinolone treated groups. All these malformations are skeletal as appeared by Alizarine red stain.

-The effects of fluoroquinolones after labor.

The effects of quinolones on neonates count after birth:

The mean number of pups delivered by treated animals in Norfloxacin , Ciprofloxacin, and Enrofloxacin treated grousp was (5.666 ± 3.502), (5.833 ± 2.786) and (5.142 ± 2.267) respectively. There was significant decrease (P<0.05) in the mean number of pups in all treated groups compared to control (table 6).

The effects of fluroquinolones on neonate weights after birth:

Means weights of pups in Norfloxacin, Ciprofloxacin and Enrofloxacin treated groups was (3.732 ± 0.504 gm.), (3.320 ± 0.383 gm.), and (3.730 ± 0.837 gm.) respectively. The means weights of pups were significant decrease (P<0.01) in all treated groups compared to control group (6.164± 0.401 gm.) (table 7).

The teratogenic effects of fluroquinolones on neonates recorded after birth:

No external malformatiosn were recorded in control group , however many external malformations were recorded in Norfloxacin , Ciprofloxacin and Enrofloxacin treated group. All these malformations are skeletal (table 8).

The effects of fluroquinolones on duration of pregnancy:

The means of duration of pregnancy in Norfloxacin , Ciprofloxacin , and Enrofloxacin treated groups was (20.625 ± 0.517 day) (21.000± 0.534), and (20.625 ± 0.517 day) respectively . However only Norfloxacin and Enrofloxacin treated group showed significant decline (P< 0.01) in the length of gestation period (table 9).

DISCUSSION

Administration of Norfloxacin (700 mg/kg/day), Ciprofloxacin (550 mg/kg/day), & Enrofloxacin (750 mg/kg/day) orally to pregnant female rats significantly decreased the litter size. These results could be attributed to the inhibitory effect of fluoroquinolones on DNA gyrase, the enzyme essential for negative super helical twisting into double

stranded DNA (a reaction that generates tension in the double helix that favors unwinding of double strands) and the catenation and decatenation of two duplex DNA circles interlocked like links in a chain (Wolfson, & Hooper, 1985, Vancutsem et al., 1990). So inhibition of DNA transcription at early stage of rapidly divided fetal cells may decrease litter size (Al-Snaffi, et al., 1997). On the other hand DNA damage induced by CPX may be attributed to its ability to releasing oxygen free radicals (Gürbay et al., 2006). It is known that Oxygen free radicals attack DNA causing mutations (Arriaga-alba et al., 2000). The reduction in number may be due to early embryonic death and an increase in the fetal resorptions ratio . The findings in this study are in agreement with the findings of manyother researchers (Friedman & Polifka 2000 & Nahum et al., 2006)), who reported teratogenic effects in women exposed to ciprofloxacin during pregnancy. Some of them (Schaefer et al., 1996) reported 4.7% incidence of congenital malformations in live-born children exposed to ciprofloxacin during the first trimester. In a company-sponsored prospective registry of 116 human pregnancies, 54% were exposed during the 1st trimester and resulted in live births. were malformed (Schaefer et al., 1996). Nahum et al., (2006) put ciprofloxacin in category C as far the relative risk of human teratogenesis by drugs used in pregnancy. FDA Pregnancy Category C means that there isn't sufficient information or there are evidence from animal studies but no confirmation of birth defects in humans (Greenfield, 2004).

Fetal growth retardation could be occurred as a result of reduction of thickness in proliferative zone of the long bones and absence of the hypertrophic zone. Stahlmann noted that fluroquinolone delayed the developmental phase of the epiphyseal growth with growth inhibition (Stahlmann 2003). Arora reported that bone and cartilage damage could be due to fluoride accumulation with repeated fluroquinolone administration (Arora 1994). So administration of flouroquinolones are not recommended in pregnancy because of their effects on growing cartilage (Katzung et al., 1998). Fluroquinolones caused defect in cartilage development and delay the ossification process. The fetotoxic effect of ciprofloxacin was observed on skeletal growth as evidenced by decrease of intact bone length in long bones of extremities (Siddiqui et al., 2010). The skeletal changes recorded in this study are similar to those described previously by others (Kim et al., 2000, 2003,2004,2005) following administration of fluoroquinolone to the pregnant rats and rabbits. These results are also in accordance with the findings of Lemus et al., (2009) that enrofloxacin and ciprofloxacin were associated with severe alterations in the development of embryo cartilage and bones.

The fetotoxicity, high resorption ratio and fetal lost ,and malformations could be attributed to the inhibition of DNA transcription in the rapidly divided fetal cells . So fluroquinolones act as DNA gyrase inhibitors and also mitotic inhibitors. The complete damage of DNA could be resulted in fetal lost or resorption , while partial damage could be induced fetal malformation. (Al-Snafi et al., 1997 , Jinang et al, 1990, Jeffry et al, 2000).

TABLES AND FIGURES:

Table (1): The effects of fluoroquinolones on litter size:

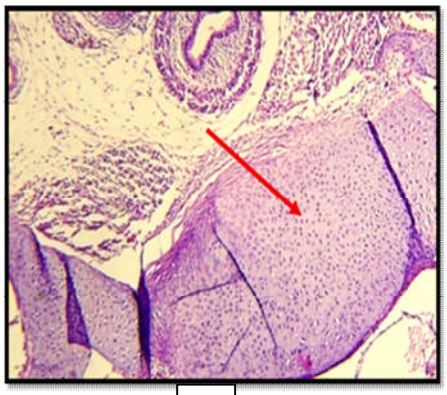
Treatment	Mean ± SD
Norfloxacin 700mg/kg/day	6.400 ± 2.302**
Norfloxacin 550mg/kg/day	6.111 ± 3.620**
Enrofloxacin 750mg/kg/day	6.555 ± 2.006**
DMS 0.5ml/animal/day	10.111 ± 1.269

**P< 0.01

Table (2): The effects of quinolones on fetal body weight :

Treatment	Mean ± SD	Number of animals
NFX(A) 700mg/kg/day	0.977± 0.179**	5
CPX(B) 550mg/kg/day	0.862 ± 0.087**	10
ENX(C) 750mg/kg/day	0.823 ± 0.405**	6
DMS(D) 0.5ml/animal/day	2.614 ± 0.140	7

**P< 0.01



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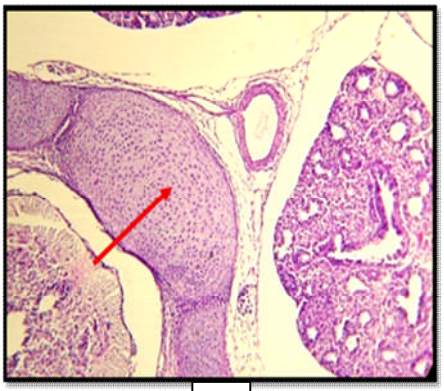

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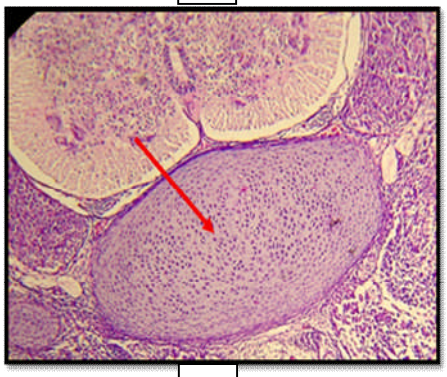

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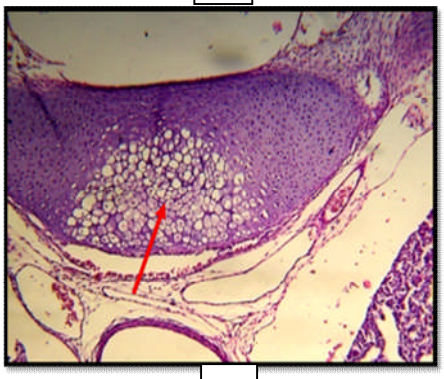

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Figure (1) : Cross section through the spinal cord and thoracic vertebrae of fetus at the day 15 of gestation from rat treated with NFX, CPX, ENX(A,B, and C), showing absence of ossification (→) in comparison with normal ossification in DMS treated group(D) . X10 H & E.

Table (3): The effects of quinolones on fetal resorption :

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	2.200 ± 0.836**
CPX(B) 550mg/kg/day	3.000 ± 2.828**
ENX(C) 750mg/kg/day	3.428 ± 1.988**
DMS(D) 0.5ml/animal/day	0.200 ± 0.632

**P< 0.01

Table (4): The effects of quinolones on fetal lost:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	2.600 ± 0.894**
CPX(B) 550mg/kg/day	2.000 ± 0.894*
ENX(C) 750mg/kg/day	3.800 ± 2.167**
DMS(D) 0.5ml/animal/day	0.250 ± 0.462

**P< 0.01 , *P<0.05

Table (5): The external fetures caused by quinolones:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	0.020 ± 0.058
CPX(B) 550mg/kg/day	0.031 ± 0.088
ENX(C) 750mg/kg/day	0.038 ± 0.0715
DMS(D) 0.5ml/animal/day	Zero

Table (6): The effects of fluroquinolones on neonates count after birth:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	5.666 ± 3.502**
CPX(B) 550mg/kg/day	5.833 ± 2.786**
ENX(C) 750mg/kg/day	5.142 ± 2.267**
DMS(D) 0.5ml/animal/day	10.555 ± 2.068

****P< 0.01**

Table (7): The effects of fluroquinolones on neonate weights after birth:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	3.732 ± 0.504**
CPX(B) 550mg/kg/day	3.320 ± 0.383**
ENX(C) 750mg/kg/day	3.730 ± 0.837**
DMS(D) 0.5ml/animal/day	6.146 ± 0.401

****P< 0.01**

Table (8): Neonatal gross malformations caused by fluroquinolones:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	0.023 ± 0.062
CPX(B) 550mg/kg/day	0.166 ± 0.372
ENX(C) 750mg/kg/day	0.061 ± 0.161
DMS(D) 0.5ml/animal/day	Zero

Table (9): The effects of fluroquinolones on duration of pregnanc/day:

Treatment	Mean ± SD
NFX(A) 700mg/kg/day	20.625 ± 0.517**
CPX(B) 550mg/kg/day	21.000 ± 0.534
ENX(C) 750mg/kg/day	20.625 ± 0.517**
DMS(D) 0.5ml/animal/day	21.666 ± 0.707

****P< 0.01**

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أجنة الفليوروكينولونات في الجرذان

أ.ج. المياحي*، أ.أ.ه. الموسوي*، علي اسماعيل السنافي**

الملخص

لقد تم تصميم هذه الدراسة للاستقصاء عن السميه الجنينيه المحتملة للكوينولينات المفلورة (النورفلوكساسين ، السبروفلوكساسين ،والانروفلوكساسين) على اجنة الجرذان . لقد تم تقسيم ٨٠ انثى جرذ حاملة الى اربع مجاميع متساوية واعطيت جرعة فمية مفردة يومياً من النورفلوكساسين ٧٠٠ ملغم /كغم والسبروفلوكساسين ٥٥٠ ملغم /كغم والانروفلوكساسين ٧٥٠ ملغم /كغم على التوالي فيما اعطيت المجموعة الاخيرة الداي مثيل سلفوكسايد ٠،٥ مل /انثى /يوم . اعطيت الادوية من اليوم الاول الى اليوم الخامس عشر من الحمل . ثم تم قتل١٠ اناث من كل مجموعة يوم ١٥ من الحمل فيما تركت العشرة اناث الاخرى لحين الولادة . وتم دراسة تأثير الادوية على حجم الحمل واوزان الاجنة ونسبة حدوث الفقد او الارتشاف الجنيني والتشوهات الخارجية .كما تم تقييم تأثير الادوية على فترة الحمل .اظهرت النتائج ان الكوينولينات المفلورة قللت حجم الحمل واوزان الاجنة بشكل ملحوظ احصائيا كما انها زادت الفقد والارتشاف الجيني مقارنه بمجموعة السيطرة عند اجراء التقييم يوم ١٥ من الحمل او بعد الولادة .

ان التقييم العياني اظهر حدوث تشوهات جنينية في الاجنة التي اعطيت امهاتها الكوينولينات المفلورة في الخمسة عشر يوم الاولى من الحمل .

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* قسم الفسلجة - كلية الطب البيطري - جامعة البصرة

** قسم الأدوية - كلية الطب - جامعة ذي قار