TOXICOLOGICAL AND HEALTH HAZARDS OF ENROFLOXACIN AS AN ANTIMICROBIAL AGENT ON REPRODUCTION OF MALE ADULT ALBINO RATS.

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ABSTRACT

This study includes determination of the toxic effect of enrofloxacin as an antimicrobial agent in doses of 250 and 500 mg/kg B.wt. on fertility parameters including some reproductive organs weight, sperm cell count, motility, sperm live and dead percentages, fructose, and testosterone level with detection of its residues in tissue and the histopathological findings on some organs.

A number of 90 adult male albino rats were classed into 3 groups each of 30 rats. First group was treated orally with 250 mg/kg B.wt. The second group was treated orally with 500 mg/kg B.wt. daily for 8 successive weeks, while the third group kept as control group. The results reveals sever inhibition on all fertility parameters which confirmed through histopathological findings, also the results concluded that enrofloxacin has residues in testis after stoppage of administration by one week .

INTRODUCTION

The use of enrofloxacin and Ciprofloxacin become have a serious problem, how ever as they are substances that leave residues in edible tissue which may be directly toxic or cause resistant pathogens and possible allergic hypersensitivity reactions in human ***(Anadone, et al.,1999).***

Fluorquinolones have undesirable adverse effects such as gastro-intestinal toxicity, photo toxicity, and developmental toxicity beside arthropathy in humans and experimental animals ***(Stahlmann and Lode, 1999).*** Moreover Ofloxacin, Ciprofloxacin, and Pefloxacin impair both testicular function and structure in rats with marked decrease in fertility. ***(Adel et al., 2000).***

Recently it was reported that fluorquinolones have adverse effects on pregnant dams and Embryo-fetal developments in rats ***(Kim et al., (2003)***.

The testicular atrophy observed in dogs at both 30 and 90 mg/ kg b.wt/d was due to the disappearance of spermatocytes and spermatogonia in somniferous tubules and the atrophy of somniferous tubules. Although the testicular atrophy was not severe, multinuclear giant cells and vacuolation in

sertoli cells were observed in semn-iferous tubules. ***(Bertino, and Fish. 2000).***

The aim of the recent work study the effect of enrofloxacin (recent Quinolone) on male rate fertility and to monitor its toxic effects on different vital organs.

MATERIAL & METHODS

Experimental animals:

In this study a ninety apparently healthy male adult albino rats weighing 120-135 gm were used .They were obtained from Lab animal colonies–ministry of public health, Helwan, Animals were housed in metallic cages (10 rats/cage) and the hygienic condition was kept constant throughout the experimental period.

Grouping of rats:

Rats were divided into three groups each of 30 rats: the 1st group was used for studying the effect of daily oral administration of 1/20 LD50 (250 mg/kg BW) as a small dose, the 2nd group 1/10 LD50of enrofloxacin 10% (500 mg/kg BW) as a large dose group, and the 3rd group kept as a control.

Dosing:

Dosing was carried out depending on the basis of oral medium lethal dose (LD50) for male albino rats as 5000 mg /kg b wt. ***(Altreuther, 1992)***, and given orally by a mean of stomach gavage daily for a period of 8 weeks in two doses levels, low dose (1/20 LD50) 250 mg/kg, and high dose (1/10LD50)500 mg/kg.

Blood sampling:-

Blood samples were obtained from each rat in each group from the orbital venous plexuses using a capillary tube after 8 weeks for revealing the different fertility studies.

Histopathological samples:

Testis, epididymis, seminal vesicle and prostate gland were immediately dissected out then prepared for histopathological examination.

Fertility studies

The effect of enrofloxacin 10% on fertility of mature male albino rat were estimated by semen evaluation, estimation of serum testosterone, also histopathological examination and detection of the drug residues in testes.

Semen evaluation:-

-Assessment of sperm count and motility

Sperm count and motility were assessed according to ***Freund and Carol. (1965).***

-Sperm alive and dead percentage

Alive and dead percentage of sperms were assessed according to ***Hancock (1952).***

-Estimation of fructose in semen

Fructose in semen was determined by using ready made kits of Biodiagnostic Company according to ***Foreman (1973).***

-Determination of testosterone level in serum

Testosterone in serum was determined by using ready made kits according to ***Greenway et al., (1983).***

-Histopathological studies of reproductive organs

At the termination of the experiment Speicement of testis , seminal vesicles, prostate glands were dissected from both control and treated groups then dried by filter paper and fixed by formalin 10% solution till used in histopathological examination. The isolated organs were processed for preparing paraffin sections of 3-5 microns thickness, the paraffin sections were stained with Ma-

yer's heamatoxilline and eosin for micr-oscopic examination according to ***Dury and Wallingiton (1980).***

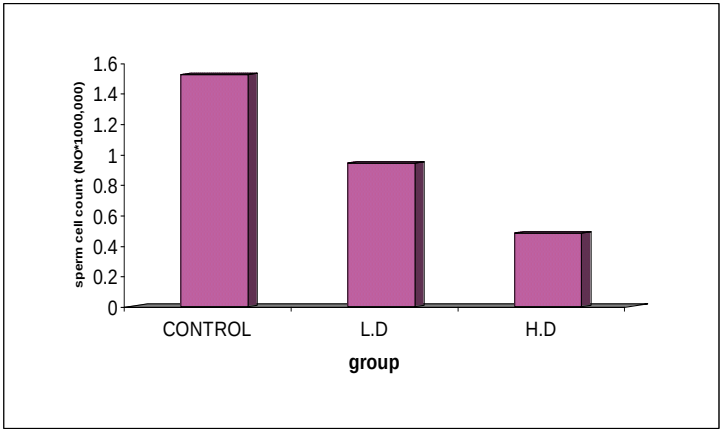
Statistical analysis:
Statistical analysis of the obtained data was carried out using **"T"** test according to ***Berly and Lindgren (1990).***

RESULTS

Table (1): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on sperm cell count (No×10 ^{6}) in semen of adult male albino rats .

Duration time 8 weeks / group	Sperm cell count (No×10^{6})
Control	1.5 ± 0.09
250 mg / kg	0.9 ± 0.11**↓
500 mg	0.5 ± 0.06**↓

* Significant at P ≥ 0.05 ** Highly significant at P≥ 0.01



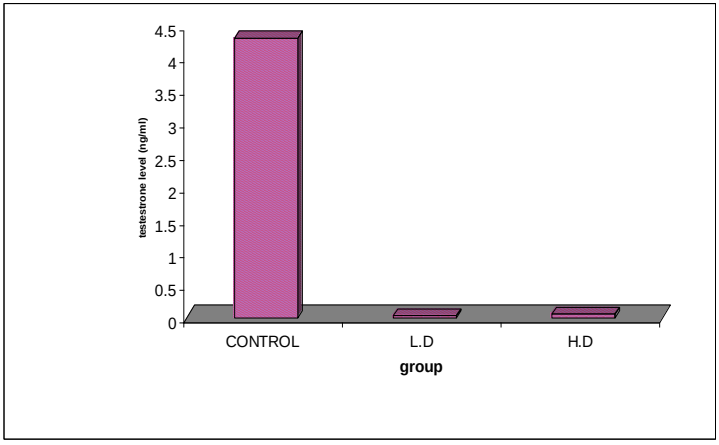
LD=low dose HD=high dose

Fig.(1): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on sperm cell count (No ×10^{6}) in semen of adult male albino rats.

Table (2): Effect of 8 weeks oral administration of enrofloxacin in doses of 250 and 500 mg/kg b.wt /day on testosterone level (ng/ml) in serum of adult male albino rats.

Duration time 8 weeks / group	Serum testosterone level (ng/ml)
Control	4.3 ± 0.4
250 mg / kg	0.03 ± 0.01**↓
500 mg	0.055 ± 0.02**↓

Significant at P ≥ 0.05 ** Highly significant at P≥ 0.01



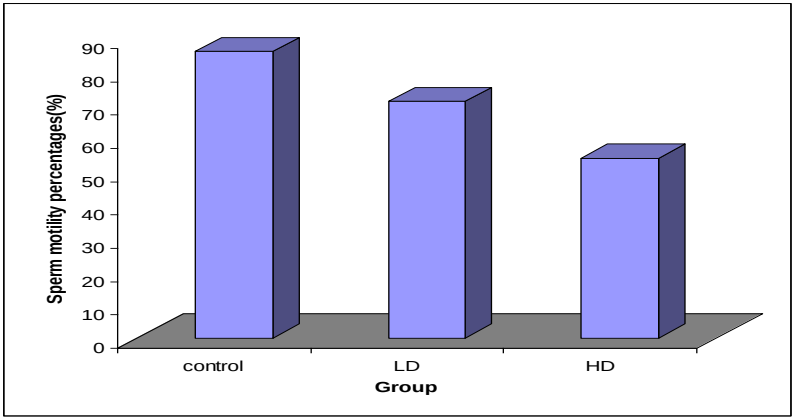
LD=low dose HD=high dose

Fig.(2): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on testosterone level (ng/ml)in serum of adult male albino rats .

Table (3): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on sperm motility,live and dead percentages in semen of adult male albino rats .

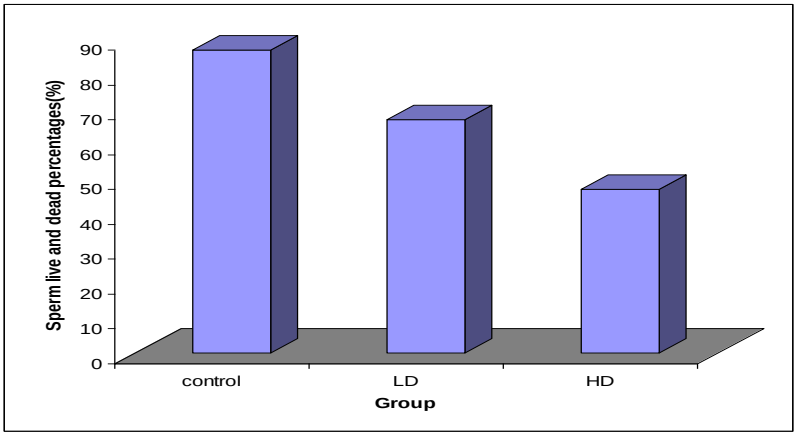
Duration time 8 weeks / group	Sperm motility percentages	Sperm live and dead percentages
Control	86	87
250 mg / kg	71↓	67↓
500 mg	54↓	47↓

*Significant at P ≥ 0.05 ** Highly significant at P≥ 0.01



LD=low dose HD=high dose

Fig.(3): Effect of 8 consecutive weeks oral administration of enrofloxacin 10% on sperm motility percentages in doses of 250 and 500 mg/kg b.wt. /day in semen of adult male albino rats.



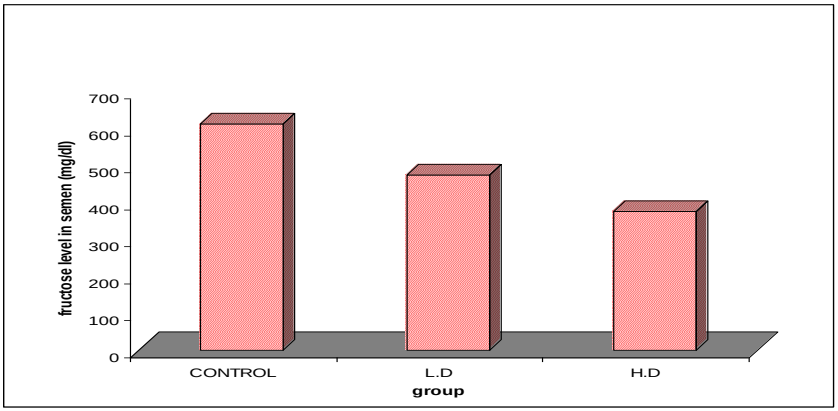
LD=low dose HD=high dose

Fig.(4): Effect of 8 consecutive weeks oral administration of enrofloxacin 10% on sperm live and dead percentage in doses of 250 and 500 mg/kg b.wt. /day in semen of rats.

Table (4): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on fructose level (mg/dl)in semen of adult male albino rats .

Duration time 8 weeks / group	Fructose level in semen (mg/dl)
Control	611.2±38.3
250 mg / kg	474.3± 34.5*↓
500 mg	375.1± 49.01**↓

Significant at P ≥ 0.05 ** Highly significant at P≥ 0.01 *



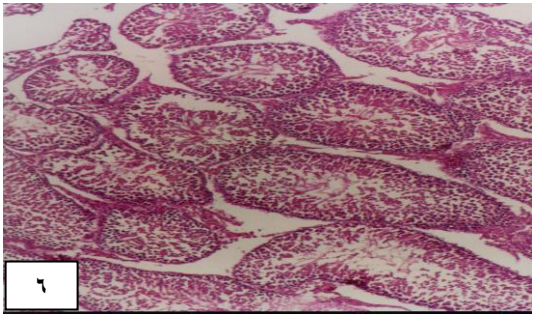
LD=low dose HD=high dose

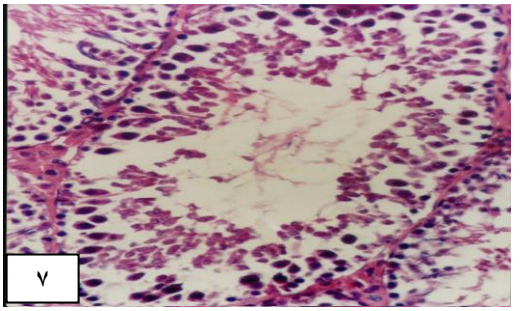
Fig.(5): Effect of 8 weeks oral administration of enrofloxacin 10% in doses of 250 and 500 mg/kg b.wt /day on fructose level (mg/dl) in semen of adult male albino rats .

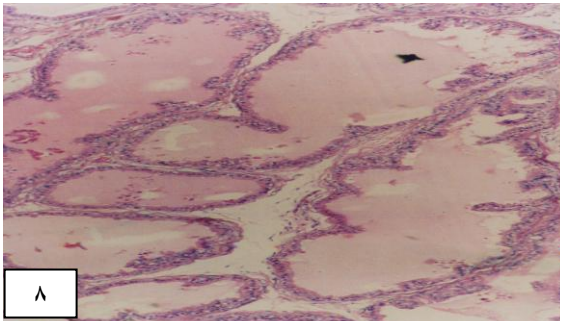
Histopathological study of reproductive organs:

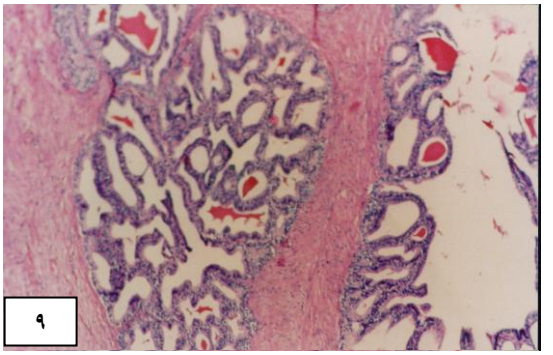
(Testes, seminal vesicles, and prostate glands).

The testes revealed marked degeneration and depletion of spermatogenic cells at dose of 250 mg/kg b wt. orally for 8 weeks **Fig.(6)**. while at dose of 500 mg/kg/b.wt /day revealed that spermatogenic tubules lack of spermatogenic cells especially in central part **Fig.(7)** The seminal vesicles showed degenerative changes in the epithelial lining with cystic dilatation at dose of 250 mg/kg b wt. orally for 8 weeks **Fig.(8).** while at dose of 500 mg/kg/b.wt /day showed degenerated epithelium with nuclear pyknosis **Fig.(9)**. prostate gland showed mild epithelial degeneration and hyperplasia at dose of 250 and 500 mg/kg/b.wt/day orally for 8 weeks **Fig.(10) .**









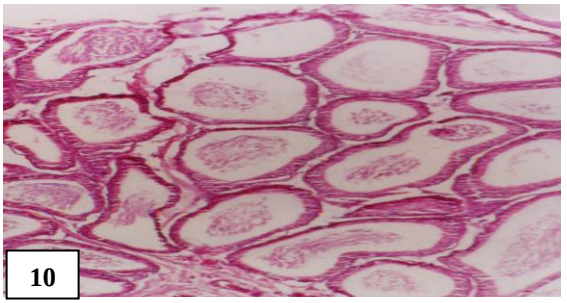


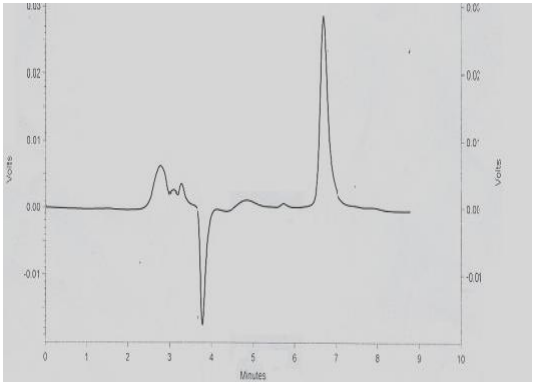
Fig. (6): Testis of rat received enrofloxacin at dose of 250mg/kg b.wt. /day for 8 weeks, Showing marked degeneration and depletion of spermatogenic cells. (H&E stain × 100).

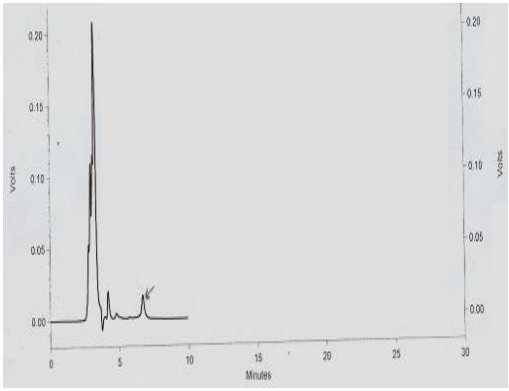
Fig. (7): Testis of rat received enrofloxacin at dose of 500 mg/kg b.wt. /day for 8 weeks, showing spermatogenic tubule that lack spermatogenic cells especially in its central Part. (H&E stain × 400).

Fig. (8): Seminal vesicle of rats received enrofloxacin at dose of 250 mg/kg b.wt. /day for 8 weeks, showing various stages of degenerating epithelium and cystic dilatation in some tubules. (H&E stain × 100).

Fig. (9): Seminal vesicle of rat received enrofloxacin at dose of 500mg/kg b.wt. /day for 8 weeks, showing mild epith-elial degeneration together with nuclear pyknosis. (H&E stain × 250).

Fig. (10): Prostate gland of rat received enro-floxacin at dose of 250 and 500 mg/kg b.wt. orally for 8 weeks, showing mild epithelial degeneration and hyperpl-asia. (H&E stain × 100).





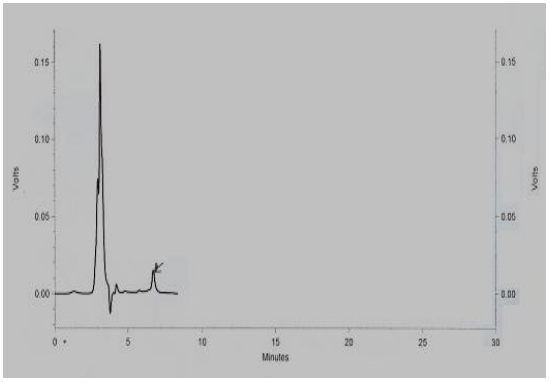


Fig.(11): Control chromatographic analysis using 0.5 ug enrofloxacin.

Fig.(12):1.8ug residues in testis of rat after 1 week stoppage of enrofloxacin oral administration at a dose of 250 mg/k b.wt for 8 consecutive weeks.

Fig.(13): 1.5 ug residues in testis of rat after 1 week stoppage of enrofloxacin oral administration at a dose of 500 mg/kg b.wt. For 8 consecutive weeks.

Enrofloxacin residues (Ug/gm) (HPLC) after stoppage of drug administration by 1 week.

Non significant trace residues of enrofloxacin were found after 1 week of stoppage of administration ranging from 1.5-2.5 ug/gm of both testis and tissue (gastrocanemous muscle).

DISCUSSION

Screening of the fertility parameters revealed significant decrease in sperm motility, live and dead percentages in both doses levels which were in agreement with ***Bare et al., (1988)*** who reported that testes appeared to be the target organ of quinolones toxicity that suppress sperm production and cause the presence of abnormal spermatozoa.

On other hand ***Schramm (1986)*** in his report about fluorquinolones toxicity found no impairment in male fertility and morphology of the testes.

These results of the of sperm motility depletion was emphasized by the work of ***Folgero et al., (1993)*** whose stated that quinolones interfere with the energy production process required for sperm vitality and motility.

There were a significant decrease of sperm cell count, testosterone and fructose level after 8 weeks oral administration of 250 and 500 mg/kg b wt.

Our results were in agreement with ***Adel et al., (2000)*** who stated that administration of ofloxacin and ciprofloxacin in rats in doses of 36.72 and 360 mg/kg b.wt /day for 15 days revealed a decrease in sperm count significantly by 10.6%, 67.5% and 97.7% respectively with respect to the control group.

These results may be attributed to its effect on spermatogenesis as it cause incomplete spermatogenesis, proliferation of interstitial leyding cells and atrophy of somniferous tubules leading to decrease of the fertility parameters ***Adel et al., (2000)***.

On other hand these results were in disagreement with those recorded by ***Porter et al., (1988)*** whose stated that following administration of enrofloxacin in doses of 0.3 or 1.2 mg/kg b.wt for 90 days to an immature age, testes and epididymis appeared mature in all treated animals and they contains normal mature spermatozoa which may be attributed to the usage of sub therapeutic dose.

Histopathological examination of some organs of rats group received enrofloxacin at dose of 250 and 500 mg/kg/b.wt/day orally 8 weeks were in agreement with ***Porter et al., (1985)*** whose reported that histopathological examination of epididymis of dog group taken fluorquinolones in

a dose of 92 mg/kg b.wt /day revealed bilateral testicular degeneration characterized by multi nucleated giant cells , cells with large cytoplasmic and nuclear volumes, and mitosis in somniferous tubules. Although for 90 days administration of 0.3, 0.6, 102 and 92 mg/kg b.wt /day. Somniferous tubules lined with single layers of spermatogonial cells and forming open lumens were present.

On the other hand these results were in disagreement with ***Porter et al., (1988)*** whose reported that following administration of enrofloxacin in doses of 0.3 or 1.2 mg/kg b.wt for 90 days to an immature age there were no gross nor microscopical changes of testes and epididymis indicated toxicity.

Our investigation concerning the residues of enrofloxacin in muscles and testes using high performance liquid chromatographic analysis after 1 week stoppage of enrofloxacin oral administration revealed the presence of residue in concentration ranged from 1.5 to 2.5 ug/gm in two muscles samples and 4 testicular tissue samples.

Despite the recorded residues levels being lower than that regulated by ***Brussels (1999),*** in edible tissues, from the toxicological point of view, great attention should be taken during dosing animals for meat production with enrofloxacin and other quinolones.

The presence of these residues may be attributed to wide range of distribution, good tissue penetration, and its long elimination half- life. ***Elmas et al., (2000).***

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الملخص العربى

المخاطر السمية والصحية للانروفلوكساسين كمضاد للميكروبات على التناسل فى ذكور الفئران البيضاء البالغة

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استهدفت هذه الدراسة البحث فى الجوانب السمية والاثار الجانبية لاستخدام عقار الانروفلوكساسين وهو احد المضادات الحيوية المستخدمة بكثرة فى مجال الطب البيطرى بهدف القضاء على بعض الميكروبات المسببة لكثير من الامراض التى تفتك بالثروة الحيوانية والداجنة وقد تم التركيز فى هذة الدراسة على تأثير استخدام هذا العقار على الخصوبة فى ذكور الفئران البيضاء البالغة.
وقد استخدم فى هذا البحث عدد تسعون من ذكور الفئران البيضاء البالغة يتراوح اوزانها بين ١٢٠-١٣٥قسمت الى ثلاث مجموعات كل مجموعة تحتوى على عدد ثلاثون فأر المجموعة الاولى استخدمت لدراسة تأثير جرعة تعادل ٢٠/١ من الجرعة النصف مميتة للعقار وهى تعادل ٢٥٠ مجم لكل كجم من وزن الجسم.
والمجموعة الثانية جرعت بضعف الجرعة السابقة اى ٥٠٠ مجم لكل كجم من وزن الجسم اما المجموعة الثالثة فكانت المجموعة الضابطة .
جرعت الفئران فى كل من المجموعة الاولى والثانية عن طريق الفم يوميا فى موعد ثابت من النهار على معدة خاوية بواسطة انبوبة اللى المعوى ولمدة ثمانية اسابيع متتالية.
تم دراسة تأثير الانروفلوكساسين على الكفاءة التناسلية فى ذكور الفئران البيضاء البالغة واظهرت النتائج ما يلى:
انخفاضاً ملحوظاً فى وزن كل من الخصيتين والبربخ والاوعية المنوية والبروستاتا .
كما اوضح البحث ان هناك انخفاضاً معنوياً فى نسب كل من عدد الحيوانات المنوية الحية وكذلك معدل الحركة ونسب كلاً من هرمون التستوسترون ونسبة الفركتوز فى السائل المنوى.
وقد ضمت هذه الدراسة البحث فى مستوى بقايا هذا العقار فى انسجة الجسم والخصية.
تم الفحص الخلوى لانسجة كل من الخصية والبروستاتا والاوعية المنوية واسفرت النتائج عن تغيرات باثولوجية جوهرية فى كل الاعضاء التى تم فحصها.
وقد اوضحت هذه الدراسة ان عقار الانروفلوكساسين له تأثيرات جانبية على الكفاءة التناسلية فى ذكور الفئران البيضاء البالغة.