The effects of ciprofloxacin on male rabbits: Biochemical and histopathological study

Ahlam A. AL-Rikaby Rashad Fadhil Ghadhban Saleh K. Majeed
Coll. of Vet. Med. / Univ. of Basra

(Received 19 January 2015, Accepted 22 June 2015)

Abstract

The present study was designed to investigate the possible effect of ciprofloxacin on blood serum enzymes activities (AST, ALT and ALP), total cholesterol, total protein, albumin and globulin as well as the histopathological changes on liver, kidney and testes. The study conducted on twelve male rabbits divided into two equal groups. G1 was administrated distilled water orally and considered as control group. G2 was given 100 mg/kg B.W/day of ciprofloxacin for 35 days orally. Blood samples were taken from animals after 35 days of treatment for biochemical analysis to estimate; total cholesterol (TC), aspartate amino-transferase (AST), alanine-aminotransferase (ALT), alkaline phosphates (ALP), total protein, albumin and globulin, then all animals were sacrificed, liver, kidney and testes were taken and fixed in 10% formalin for histological examination. The results showed that ciprofloxacin administration significantly ($p< 0.05$) increased the serum total cholesterol, AST, ALT and ALP concentration. While there were significant ($P<0.05$) decreased the serum level of total protein and globulin, but no significant changes was observed on the serum level of albumin. While histopathologically the liver show vacuolation of hepatocytes and congestion of central vein. The kidney display vacuolation of cortical areas and dilatation of tubules. While the testes exhibits suppression of spermatogenesis in treated animals compared with control group. These results revealed the toxic effect of ciprofloxacin on liver, kidney and testes of male rabbits.

Key words: Ciprofloxacin, biochemical tests, histopathology, male rabbits.

التأثيرات المرضية النسجية والكيموحيوية للسبروفلوكساسين في ذكور الارانب

احلام علي الركابي رشاد فاضل غضبان صالح كاظم مجيد
كلية الطب البيطري / جامعة البصرة

الخلاصة

اجريت هذه الدراسة لتقييم تأثير السبروفلوكساسين على بعض الاختبارات الكيموحيوية وكذلك نسيج الكبد ، والكلية والخصية في ذكور الأرانب.استخدم 12 أرنبا قسمت عشوائيا الى مجموعتين متساويتين المجموعة الأولى سيطرة سالبة وأعطيت ماء مقطرعن طريق الفم والمجموعة الثانية معاملة وأعطيت السبروفلوكساسين بجرعة 100 ملغم/كغم من وزن الجسم يوميا عن طريق الفم ولمدة 35 يوما. وبعد 35 يوما تم سحب عينات من الدم ثم تم تضحية الحيوانات لأخذ الأعضاء لغرض أجراء الفحص النسجي. أظهرت النتائج ارتفاع معنوي في الكوليسترول الكلي وإنزيمات الكبد بينما اشارت الدراسة الى انخفاض معنوي في بروتينات الدم (البروتين الكلي والكلوبيولين) ولكن انخفاض غير معنوي في الالبومين عند مقارنته مع مجموعة السيطرة. وأظهر الفحص النسجي تغيرات نسجية في مجموعة المعاملة وجود احتقان وتفجي في خلايا الكبد وتوسع الانيبيبات الكلوية وعدم تكوين الحيامن مقارنة مع مجموعة السيطرة وهذه النتائج أظهرت ان العقار له تأثير سمي على نسيج الكبد والكلية والخصية مقارنة مع مجموعة السيطرة.

الكلمات المفتاحية: سبروفلوكساسين ، الاختبارات الكيموحيوية ، نسيجية مرضية ، الكلية ، الخصية ، ذكور الأرانب.

Introduction

Ciprofloxacin (CPX) is a synthetics antibacterial agent; it was discovered at the end of 1982 by Bayer. Ciprofloxacin is second generation of fluoroquinolons group

with a very broad spectrum of bacteria transcription especially gram negative infection disease (1). It is used in a variety of human clinical infection like urinary tract, bone and soft tissue, skin, respiratory tract, reproductive tract gastro-intestinal infection (2). It is well absorbed orally, good to excellent tissue penetration and induced its antibacterial action mainly by inhibition of DNA gyrase, which is equivalent topoisomerase II in mammalian (3). Ciprofloxacin exert it action by blocking bacterial DNA synthesis through inhibition bacterial topoisomerase IV. Inhibition of DNA gyrase prevents the relaxation of positively supper coiled DNA that is required for normal transcription (4). Inhibition of topoisomerase interferes with separation of replication chromosomal DNA in to respective daughter cell during cell division (5,6). Who found that quinolones antibiotics inhibit eukaryotic as well as prokaryotic cell growth and protein synthesis by interfering with DNA and RNA replication. In vivo study that ciprofloxacin produced significant enhancement of lipid peroxidation and alteration of glutathione redox status in hepatic tissue in rats (7). On the other hand, some researchers suggested that free oxidative radical generation could induce oxidative stress in the liver leading to organ damage. This is supported reported increased in lipid hydro peroxide (LOOH) in the liver by ciprofloxacin which is a marker of induced oxidative stress in liver of mouse (8). The formation of free radicals by ciprofloxacin in microsomal system might provide an explanation for the mechanism of adverse effects observed after administration of this drug to patients. The mechanism of radical formation by ciprofloxacin might be a result of metabolism of this drug by cytochrome P450 and / or redox reaction. It was reported that the preferential zone-3 distribution of hepatic damage, suggests a possible involvement of the cytochrome P450 enzyme. The enzyme activity is highest in zone-3 and it has been shown that ciprofloxacin suppresses relevant cytochrome P450 at the transcription level (9). (7) studied the effect of ciprofloxacin on foetal hepatocytes and found that the number of hepatocytes showed a marked decreased per unit area while their size increased with decrease nuclear size which may be attributed to fat deposition and interference with RNA and DNA protein synthesis in response to toxic effect of ciprofloxacin. The current study was done to evaluate the effects of ciprofloxacin.

Materials and methods

Twelve, healthy male rabbits (*Lepus curiculus*) weighing (1-1.5 kg) and aged six months were used in this study. Animals were obtained from local market in Basra city. They were housed in cages under controlled condition of temperature 25±2°C and 12 hrs. light/dark cycle. They were fed a standard pellet and drinking water adds libitum. They were left for two weeks for acclimatization before starting the experiment. The rabbits were weighed and divided randomly into equal groups of 6 rabbits each as follows. Group I: (Control). The animals were considered as control and they received distilled water for 35 days orally. Group II: (Treated) This group of rabbits was received ciprofloxacin (100 mg/kg. B.W) for 35 days orally. The ciprofloxacin was dissolved in distilled water and introduce as single daily dose administered by gastric intubation technique.

Blood collection: After 35 days the blood samples were collected from the heart of rabbit and serum separated by centrifuge at 3000 rpm for 10 minutes. The serum used for some biochemical measurements was done by using special enzymatic kits.

Biochemical assay: The serum total cholesterol (TC) was determined by using commercial kits (CHOD-PAP/Biolabo reagents, France). While the alanine aminotransferase (ALT) and aspartate amino-transferase (AST) activities were determined by using the commercial method (Biolabo reagents, France) test kits, the absorbency was determined at 505 nm wave length against reagent blank (10). The alkaline phosphatase activity (ALP**)** was assayed by using commercially kit. The read absorbance at 510 nm wave length against reagent blank

(11).The serum total protein were determined by the Biuret method using commercial kits (12). While albumin value was obtained by Bromocresol green method (Biolabo reagent, France) test kits. The globulin was determined to the method close (13).

Histological preparation: Animals were sacrificed after 35 days. The liver, kidney and testes were excised and cleaned with normal saline and immediately fixed in the 10% formalin, dehydration in ascending series of ethanol, cleared in xylene then embedded in paraffin wax. The specimens were sectioned at 5 microns thickness and stained with Hematoxyline and Eosin (H&E) (14).

Statistical analysis: The results of the present study were analyzed by using one-way analysis of variance (ANOVA) test. The data were expressed as a means ± SE, $P<0.05$ were considered to be statistically significant (15).

Results

Results were show a significant ($P<0.05$) increase in serum levels of AST, ALT, ALP and total cholesterol level in animals treated by ciprofloxacin when compared with control group (Table 1). The treatment with ciprofloxacin cause a significant ($P<0.05$) decrease in serum level of total protein and globulin, while there was no significant change in the albumin level when compared to the control group (Table 2).

Table (1): Serum AST, ALT, ALP and total cholesterol (TC) levels of control and ciprofloxacin treated groups.

Groups	AST unit/L	ALT unit/L	APL unit/L	TC mg/dl
C	24.69 ±0.44 B	63.07 ±1.31 B	67.31 ±0.79 B	144.95 ±1.03 B
T	32.19 ±0.50 A	71.45 ±0.60 A	75.29 ±0.90 A	197.77 ±0.61 A

C=Control, T=Treated: The different letters means statistical differences (P<0.05) in compared with control.

Table (2): Serum total protein, albumin and globulin levels of control and ciprofloxacin treated groups.

Groups	Total protein mg/dl	Albumin g/dl	Globulin g/dl
Control	6.23±0.12 B	3.85±0.09 A	2.22±0.16 B
Treated	5.28±0.13 A	4 ±0.17 A	1.25±0.09 A

The different letters means statistical differences (P<0.05) level in compared with control.

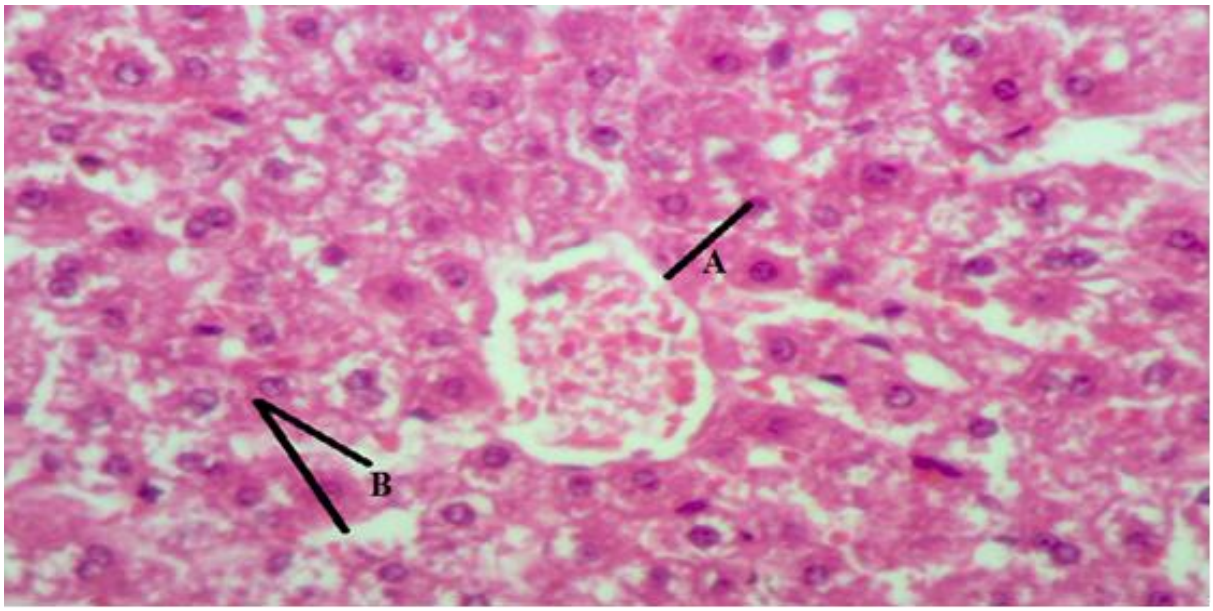

Fig. (1): Liver of rabbit treated with ciprofloxacin (100mg/kg B.W) for 35 days orally. Note there is congestion of central vein (A), and vacuolation of hepatocytes (B) (H&E X400).

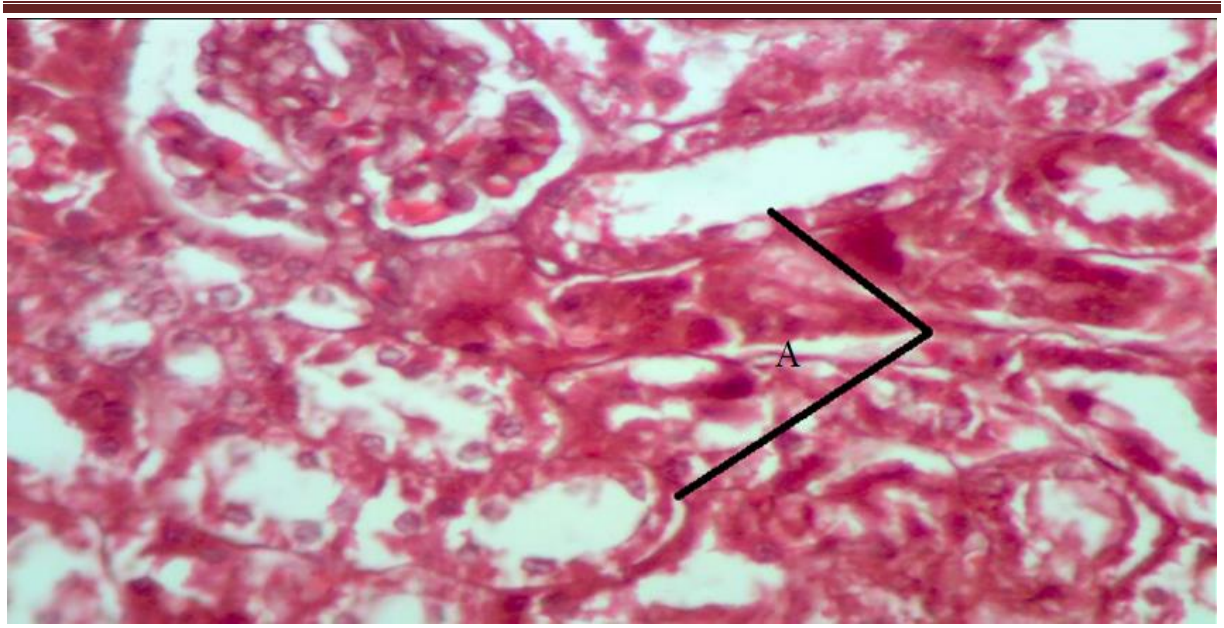

Fig. (2): Kidney of rabbit treated with ciprofloxacin (100mg/kg B.W) for 35 days orally. Note there are cortical areas of vacuolated and dilated tubules (A) (H&E X400).

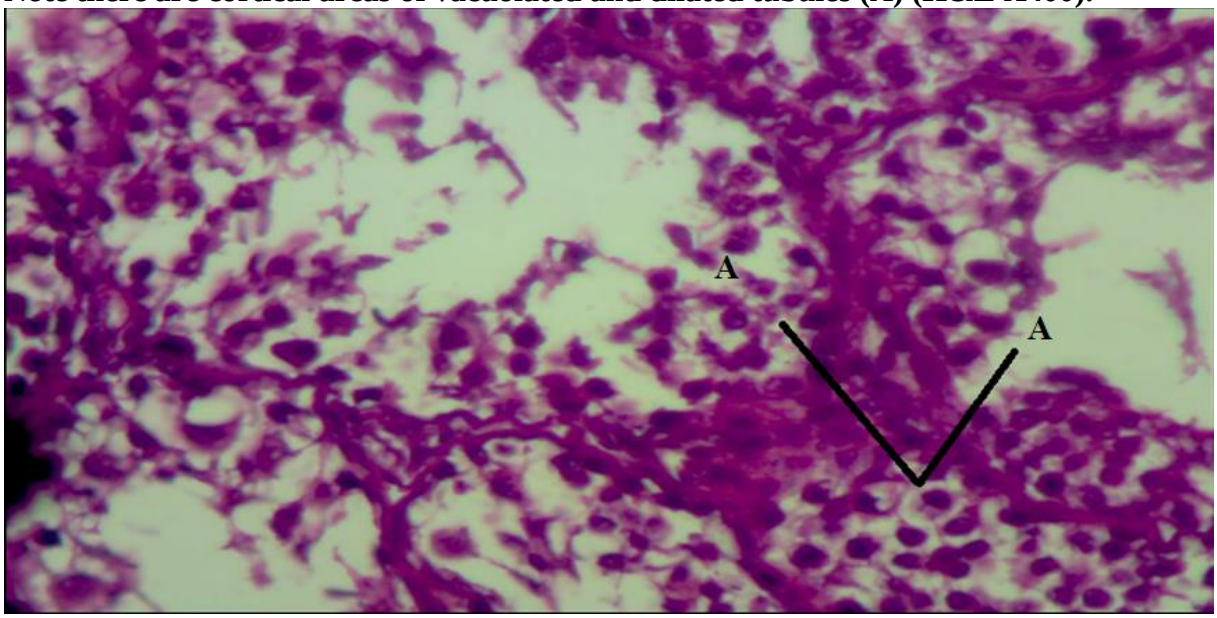

Fig. (3): Testes of rabbit treated with ciprofloxacin (100mg/kg B.W) for 35 days orally. Note there are seminiferous tubules with suppression spermatogenesis only spermatogonia (A) (H&E X400).

The histo-pathological alteration were observed in the liver from male rabbits treated with100 mg/kg of ciprofloxacin show presence of vacuolation of hepatocyte and congestion of central vein (Fig. 1). The kidney shows vacuolation of cortical areas and dilatation of tubules (Fig. 2). While in the testes seen suppression spermato-genesis and presence of spermatogonia (Fig. 3)

.

Discussion

Ciprofloxacin is known to be one of the best drugs for the treatment for many bacterial infections. The biochemical alteration observed in the present study is in parallel with the histological finding. Results are revealed a marked increase in AST, ALT and ALP in rabbit treated with ciprofloxacin. This is agreed with the finding by (16)

observed that ciprofloxacin induced liver injury marked with elevated liver enzymes (AST, ALT and ALP). (17) Demonstrated that ciprofloxacin induced hepatotoxicity in most patient treated with therapeutic doses is characterized by elevated levels of AST, ALT and ALP. This is supported by (18) who found that ciprofloxacin induced hepatic failure, hepatitis, cholestatic jaundice and acute liver injury marked by elevated level of liver enzymes due to the ciprofloxacin has a potential hepatotoxic agent. From the statistical increase in total cholesterol in treated group as compared with control, it could signify a high risk of heart disease or blood vessel disease. This agrees with finding of (19 and 20) who reported that ciprofloxacin associated cardio toxicity and hepatotoxicity. The results revealed a marked decrease in protein contents of liver in treated male rabbits with ciprofloxacin. The hepatotoxic effect of ciprofloxacin may be due to oxidative stress induced in the liver by ciprofloxacin through the generation of oxidative radicals leading to depletion of protein content in hepatocytes. The finding is in agreement with (21) demonstrated the depletion of protein content is a consequence of nucleic acid diminution and the damage of DNA leading to a significant decrease in the number and degeneration in mitochondria which is responsible for energy supply. This statement is further supported by the fact that ciprofloxacin antibacterial activity has been ascribed to DNA binding resulting in a marked inhibition of bacterial DNA topoisomerase (22 and 23). (6) who found that the quinolone antibiotic inhibits protein synthesis by interfering with DNA and RNA replication. (24 and 25) reported that the DNA damaging effect of quinolones in liver and kidney may be due to the fact the organs play a major role in the metabolism and excretion of quinolones. (26) Came to the conclusion that protein depletion is a consequence of nucleic acid diminution. The present study indicated that administration of ciprofloxacin induced various changes in the liver of male rabbits, these changes varied from vacuolation of hepatocytes, congestion of central vein and vacuolation of the centrilobular region. The quinolones are very important antimicrobial because it effect on a wide variety of aerobic organisms (27) but the result obtained from work where similar finding is reported by (28) Who found that ciprofloxacin has the potential to induce hepatotoxicity and renal toxicity effects. Liver damage is previously observed (29) who found that ciprofloxacin treated caused congestion of sinusoids, centrilobular necrosis and inflammatory leucocytic infiltration. In support of this hypothesis the administration of therapeutic and double therapeutic doses of ciprofloxacin (57mg and 114mg/kg) in two periods (pre implantation and post implantation of pregnancy) induced various change in liver of pregnant rats and their fetuses (30). Similar observation by several authors following ciprofloxacin treatment (31, 32 and 33) who showed that the ciprofloxacin caused hepatocellular necrosis and mixed inflammation infiltrate in livers of patients. In addition the kidney of the rabbits treated with ciprofloxacin showed also vacuolation and dilation of cortical renal tubules. This result agrees with the finding of (34) who found that administration of ciprofloxacin induced cell swelling of the epithelial lining of renal tubules and necrosis in kidney of the juvenile rats. In present study, administration of ciprofloxacin caused suppression spermatogenesis and present spermatids in the lumen of seminiferous tubules. This in concordance with the finding by (35, and 36) concluded that ciprofloxacin treatment caused in a marked reduction in sperm count and motility the diminution of these sperm may be referred to the interference ciprofloxacin with energy production process required for sperm vitality and motility. Some reports (37, and 38) stated that ciprofloxacin treatment in rats significantly impair testicular function and structure. These results indicate that ciprofloxacin like other chemical agents may directly interfere in the process of spermatogenesis this may due to an increased peroxide radical generation in the testes following ciprofloxacin treatment. In conclusion: According to the results obtained from this study. Ciprofloxacin has potential

hepatotoxicity as evidenced by increased enzymes activities (AST, ALT, ALP), total cholesterol, total protein, albumin and globulin, nephrotoxicity and testicular toxicity as evidenced by suppression spermatogenesis at dose (100 mg/kg B.W) in male rabbits.

References

1-Sub B, Lorber B (1995) Quinolones. Med. Clin. 79: 869-894.

2-Hooper DC, wolfson JS (1985) The fluoroquinolones pharmacology clinical uses toxicities in human. Antrimicro. Agen. Chem. 28(5): 716-721.

3-Liu Q, Wong JC (1999) Similarity in the catalysis of DNA break age and rejoining by type IA and IIA DNA topoisomerase. Proc. Nalt. Acad. Sci. USA. 96:881-886.

4-Lica K, Zhao X (1997) DNA gyrase topoisomerase Iv and the 4-quinolones. Microl. Mol. Bio. Rev. 61 (3):377-392.

5-Laurence LB, Parker KL (2008) Ciprofloxacin in chemotherapy of microbial disease. Acad. Press. New York . 709-839.

6-Minuk GY, Assy N, Ding LX (1997) Effect of quinolone antibiotics on hepatic growth and protein synthesis following partial hepatectoomy in rats. J. Gastro. Haptol. 12: 5-7.

7-Channa MA. Janjua MZ (2003) Effect of Ciprofloxacin on fetal hepatocytes. J. Pak. Med. Assoc. 53 (10): 448-450.

8-Weyers AI, Ugma LI, Carcia OH, Gloria GB (2002) Ciprofloxacin increases hepatic and renal lipid hydroperoxide levels in mice. Biocell. 26(2): 225-228.

9-Xie HJ, Brombery GL, Rane AH, Lundgren SC (2003) Alteration of pharmacokinetics of cyclophosphamide and Suppression of the cytochrom P450 genes by Ciprofloxacin. Bon. Marr. Trans. 31: 197-203.

10-Khuon OS (2012) Pharmacological effect of aqueous leaves extract of Ocimum basilicum against liver toxicity induced by acetaminophen in male rats. J.Agric.Res.1(1):13-28.

11-AL-Mousa H, AL-Hashem F (2014) Hypoglycemic, Hepato-renal and antioxidant potential effect of Chamomile recutita flowers ethanolic extract in streptozotocin-diabetic rats. Amer. J. Pharma. Toxicol. 9(1): 1-12

12-AL-Mousawi ZAH (2013) Effect of vitamin C and zinc on some biochemical parameter in alloxan induced diabetic rabbits. Bas. J. Vet. Res.12(1):77-86.

13-Close EH (1986) Veterinary Clinical Pathology 4th ed. W.B. Saunders company. Philadelphia, USA.

14-Bancroft JD, Stevens A, Turner DR (1996) Theory and Practice of histological techniques 4th ed. New, York, Churchill, Living, stone.

15-Saeys y, Inza I, Larraaga P (2007) A review of future selection techniques in bioinformatics. Bioinformatics. 23:2507-2517.

16-Orman SE, Conjevaram HS, Rochen J (2001) Clinical and histological features of fluoroquindones induce liver. Injury. Clin. Gastrol. Hepatol. 3: 517-523.

17-Bhagirath MK (2008) A case report of high suspected Ciprofloxacin-induced heatotoxicity. Tur. J. Gastro. 19(3):204-206.

18-Hirsch A, Lundquist LM (2009) Ciprofloxacin–induce hepatotoxcity resolved with lovefloxcin. Hosp. Pharm. 44(11):978-983.

19-Saracoglu A, Temel HE, Ergun B, Colak O (2009) Oxidative stress medicated myocardiotoxicity of ciprofloxacin and ofloxacin in juvenile rats. Drug Chemical Toxicology. 32(3): 238-242.

20-Cholongitas E, Georgousaki C, Spyrou S, Deisenaki M (2009) Ciprofloxacin induce acute cholestatic hepatitis., Annals of Hepatology. 83(4): 400-401.

21-Gurbay A, Gonthier B, Daveloose O, Favier A (2001) Microsoma metabolism of Ciprofloxacin generates free radicals. Fre. Rad. Bio. Med. 30(10): 1118-1121.

22-Rawi SM, Mourad IM, Arafa NMS, ALazabi NI (2011) Effect of ciprofloxacin and levofloxacin on some oxidative stress parameters in brain regions of male albino rats.Afric.J. Pharma. Pharmacol.5 (16):1888-1897.

23-Gilman AG, Rall TN, Nies AS. Taylor P (1990) The pharmacological basis of therapeutics 8th Ed. Pergamon, New York. 1057-1060.

24-Contreas MA, Luna R, Mulero J, Andreu JL (2001) Severe ciprofloxacin–induced acute hepatitis. Eur. J. Clin. Microbiol. Infect. Dis. 20: 434-435.

25-Gorla N, Garcia OH, Larripa I (1999) Chromosomal aberrationsin human lymphocytes exposed in vitro to enrofloxacin and ciprofloxacin. Toxicol. Let.104:43-48.

26-Hanafy S (2000) Histological and histochemical changes in the rat kidney due to ischaemia. J. Egypt. Ger. Soc. Zool. 33(C):31-49.

27-Dana EK, Robb M, Sandra HL (2001) New classification and update on the quinolone antibiotics. Amer. Fam. Physic.

28-Adikwu E, Brambaifam N (2012) Ciprofloxacin cardio- toxicity and hepatoxicity in human and animals. Pharmcol. 3: 207-213.

29-Basaran A, Basaran N, Cunes HV, Tomati AG (1993) Effect of ciprofloxacin on chromosomes and hepatic and renal functions in rats. Chemoth. 39(3):182-188.

30-Ismait NH (2006) Assessment of histopathological and histochemical changes in liver of pregnant female rats and their fetuses following

Ciprofloxacin administration. J. Egy. Soc. Toxcol. 35: 7-17.

31-Bataille L, Rahier J, Geubel A (2002) Delayed and prolonged cholestatic hepatitis with ductopenia after long-term ciprofloxacin therapy for crohns disease. J. Hepatol. 37: 696-699.

32-Goetz M, Galle PR, Schwarting A (2003) Non-fetal acute liver injury possibly related to high-dose Ciprofloxacin. Eur. J. Micro. Infect. Dis. Hum. Rep. 22: 294-296.

33-Zimpfer A, Propst A, Mikus G, vogel W (2004) Ciprofloxacin –induced acute liver injury. Vir-Chows Arch. 444(1): 87-89.

34-AL- Showi NN (2012) Possible histological changes induced by therapeutic doses of ciprofloxacin in liver and kidney of juvenile rats. Pharmcol. 3 (9): 477-480.

35-Folgero BT, Lindal S, Oian P, Torbergsen T (1993) Mitochondrial disease and reduced sperm motility. Hum. Rep. 8: 1863-1868.

36-Zobeiri F, Sadrkhanlou RA, Salami S, Mardani K, Ahmadi A (2012) The effect of ciprofloxacin on sperm DNA damage, fertility potential and early embryonic development in NMRI mice. Veterinary Research Forum. 3 (2): 131 – 135.

37-Abd-Allah AR, Aly HA, Abdel-Aziz AA, Hamada FM (2000) Adverse testicular effect of some quinolone members in rats. Pharm. Res. 41: 211-219.

38-Dermir A, Turker P, Onel FF, Findik A (2007) Effect of experimentally induce escherichia coli epididymo- orchitis and ciprofloxacin treatment on rat spermatogenenesis. Int. J. Ural. 14: 268-272.