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Some Biochemical and Histopathological Changes in Liver of Pregnant Female Rats Following Fluoroquinolones Administration

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Abstract

The present study was designed to investigate the possible effects of fluoroquinolones (Norfloxacin, Ciprofloxacin, and Enrofloxacin) on some biochemical and histopathological changes of liver in pregnant female rats (*Ratus norvigicus*). 40 pregnant rats weighing 175-185 gm, and 10-12 weeks old in age, were divided equally in to control and three experimental groups. Control group treated with dimethylsulphoxide (DMSO) at a dose 0.5 ml/animal/day while the experimental animals treated with oral doses of (700 mg/Kg b.w. of Norfloxacin, 550 mg/Kg b.w. of Ciprofloxacin, and 750 mg/Kg b.w. of Enrofloxacin, all groups were administered these substances from the day 1 till the day 15 of gestation. Dissection was performed on day 15 of gestation after 4 hours from the last dose. Results revealed a significant ($p<0.05$) decrease in total protein content in serum of the treated groups (NFX, CPX) when compared with control, while ENX treated group showed a decrease in total protein but not significant. Assessment of globulin in all treated groups showed a significant decrease as compared with the globulin of control. Histopathological changes in liver sections of these females were in the form of dilatation of central and portal vein, and sinusoidal spaces, congestion in blood vessels, degenerated hepatocytes with necrotic nuclei. These results revealed the toxic effects of fluoroquinolones on the livers of pregnant female rats.

Key Words: Histopathological – Biochemical – Fluoroquinolones**.**

بعض المعايير الكيمياحيوية و التغيرات النسيجية في أكباد إناث الجرذان الحوامل بعد المعالجة بالكوينولينات المفلورة

الخلاصة

صممت هذه الدراسة للكشف عن التأثيرات المحتملة للكوينولينات المفلورة (النورفلوكساسين، والسبروفلوكساسين، والانروفلوكساسين) في بعض المعايير الكيمياحيوية والتغيرات النسيجية للإناث الحوامل في الجرذان المختبرية. قسمت ٤٠ من إناث الجرذان من النوع النرويجي الأبيض بوزن (١٧٥-١٨٥ كغم) وبعمر (١٠-١٢) أسبوع قسمت إلى مجموعة سيطرة وثلاث مجاميع اختبارية. أعطيت مجموعة السيطرة بمادة ثنائي اوكسيد سلفات المثيل وبجرعة ٠.٥ مل لكل حيوان في اليوم الواحد عن طريق الفم(وهي من المركبات العضوية الواسعة الاستعمال لإذابة المواد العضوية بالإضافة إلى الماء وليس لها أي تأثير سلبي) ، بينما المجاميع الاختبارية الثلاثة الأخرى فقد أعطيت جرع يومية من (النورفلوكساسين ٧٠٠ ملغم/كغم، والسبروفلوكساسين ٥٥٠ ملغم/كغم، والانروفلوكساسين ٧٥٠ ملغم/كغم) من اليوم الأول للحمل ولغاية اليوم الخامس عشر منه، ثم قتلت تلك الحيوانات في ذلك اليوم بعد ٤ ساعات من آخر جرعة ،أظهرت النتائج انخفاضا معنويا ($p<0.05$) في نسب بروتينات الدم الكلية لمصول دماء الحيوانات المعاملة بالنورفلوكساسين والسبروفلوكساسين عندما قورنت بمجموعة السيطرة، بينما مجموعة الانروفلوكساسين قد أظهرت انخفاضا في نسب البروتين الكلي لمصل الدم مقارنة بمجموعة السيطرة ولكن لم يكن معنويا. اظهر تقدير الكلوبيولين انخفاضا معنويا ($p<0.05$) في جميع المجاميع المعاملة عندما قورنت مع مجموعة السيطرة. أما نسيجيا فقد أظهرت نماذج أنسجة الكبد المأخوذة من الحيوانات المعاملة توسع الأوردة والجبيبات الكبدية،واحتقان في الأوعية الدموية الكبدية، وتنخر في خلايا الكبد وتتكز أنويتها واحتقان الأوردة البوابية، من الممكن استنتاج أن تلك العقاقير لها تأثير سمي على بيوكيميائية الدم ونسيج الكبد في إناث الجرذان الحوامل.

Introduction

Fluoroquinolones, such as Norfloxacin (NFX), Ciprofloxacin (CPX), Enrofloxacin (ENX), Ofloxacin (OFX) and Pefloxacin (PFX) represent an important class of antimicrobial agents used in treatment of a wide range of infectious diseases in different organs such as urinary tract, bone and joint, lower respiratory tract and skin (Lietman, 1995).Norfloxacin (NFX)(1- ethyl -6- fluoro - 1,4- dihydro-4-oxo-7-(1 piperazinyl)-3- quinoline carboxylic acid), is a synthetic, broad spectrum antibacterial fluoroquinolone for oral, and injection administration. It has activity against the common pathogenic gram-negative organisms that cause urinary tract infections, including Enterobacter, *Pseudomonas aeroginosa*, and Neisseria species (Katzung and Trevor, 2008). Ciprofloxacin (CPX) (1- Cyclopropyl -6- Fluoro-1, 4- Dihydro -4- Oxo -7- (1- Piperazinyl) -3- Quinolone carboxylic Acid) is an extended spectrum antimicrobial drug belongs to fluroquinolones (McKellar *et al.,* 1999). This drug is a second generation fluoroquinolones, having great activity against gram-negative bacteria and is also active against the gonococcus, many gram-positive cocci, mycobacteria, and agent of atypical pneumonia such as *Mycoplasma pneumonia*, and *Chlamydophila pneumonia* (Katzung and Trevor, 2008). Enrofloxacin (ENX) (1-Cyclopropyl- 7 - (4-ethyl-1-piperazinyl)-6-Fluoro-1,4- Dihydro -4- Oxo -3 Quinolonecarboxylic Acid) is a synthetic, broad spectrum antimicrobial medication belongs to the fluoroquinolone group of antibiotics (Wolfson, and Hooper, 1989). Enrofloxacin , a bactericidal antibiotic is 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). In many countries enrofloxacin is being used as the routine choice to treat almost any bacterial disease in poultry (Sumano and Gutierrez, 2000, 2001). CPX is the main active metabolite of ENX (Vaccaro *et al.*, 2003). The fluoroquinolones interfere with bacterial DNA synthesis by inhibiting topoisomerase II (DNA gyrase), especially in gram-negative organisms, and topoisomarase IV, especially in gram-positive organisms. They block the relaxation of super coiled DNA that is catalyzed by DNA gyrase, a step required for normal transcription and duplication. Inhibition of topisomeras IV by fluoroquinolones interferes with the separation of replicated chromosomal DNA during cell division (Katzung and Trevor, 2008). Several cases of ciprofloxacin associated severe liver damage were reported. In such cases liver biopsy revealed extensive hepatocellular necrosis and a mixed inflammatory infiltrate with abundant eosinophils in livers of patients (Contreras *et al.*, 2001; Bataille *et al.*, 2002; Goetz *et al.*, 2003; Xie *et al.*, 2003 and Zimpfer *et al.*, 2004). Hussy *et al.* (1986) suggested that fluoroquinolones may exert an inhibitory effect on eukaryotic DNA topoisomerase III resulting in the suppression of DNA synthesis. Several quinolone antibiotics, including ciprofloxacin were assayed in the in *vitro* hepatocyte primary culture/DNA repair test. McQueen and Williams (1987) reported that these compounds yielded positive results in the in *vitro* assays, but ciprofloxacin had negative results in the in *vivo* assays. In addition mammalian DNA synthesis by the polymeraseprimase complex was inhibited by high concentrations of quinolones (>100mg /L), but to a greater extent by ciprofloxacin and norfloxacin than by ofloxacin. Pino *et al.* (1991) have investigated that norfloxacin for DNA damage in rat livers and kidneys after oral administration. Maura and Pino (1988) showed that, after oral administration of quinolones, they are susceptible to be activated, presumably in the liver, to stable intermediates, which may be transformed in other organs into final reactive species interacting with DNA. Minuk *et al.* (1997) found that the quinolone antibiotics inhibit eukaryotic as well as prokaryotic cell growth and protein synthesis by interfering with DNA and RNA replication. Positive results were also observed in cytogenetic studies in *vitro* and in *vivo*, unscheduled DNA synthesis and alkaline elution tests (Gorla *et al.*, 1999). Abd-Allah *et al.* (2000), Abdo llahi and Isazadeh (2001) and Kashida *et al.* (2002) mentioned that ofloxacin induced its antibacterial action mainly by inhibition of DNA gyrase in rat and mice, which is equivalent to topoisomerase II in mammalin cells. Because these drugs are used for treatment urinary tract infection UTI in pregnant women particularly in the first trimester the present study was done to evaluate the effects of these drugs on some biochemical parameters and istopathological changes in liver of pregnant females rats.

Materials And Methods

Forty adult virgin female rats weighing (17٥-18٥ gm) were obtained from the animal house of College of Education in Thi-Qar University, Iraq. After 2 weeks period of acclimatization, cage and maintained under suitable conditions of temperature and humidity and a 12:12 light/dark cycle. Water and food were available

ad libitum.The females in proestrus phase were placed in cages overnight with untreated males (1 male to 2 females). Each morning vaginal washings were taken using distilled water and placed on microscope slides with a drop of methylene blue solution. Females showing sperm-positive vaginal smears were designated at gestational day 1. Pregnant female rats were arranged into 4 groups: the first group represented the control and received DMSO 0.5 ml/ animal/day, and the other 3 groups received oral (NFX, CPX, and ENX) by gastric intubation. The daily doses given were 700 mg/kg of NFX , 550 mg/kg of CPX and 750 mg/kg of ENX all doses were given from the day 1 till the day 15 of pregnancy, the control and treated females were sacrificed under anesthesia.The parameters had been taken (total serum protein, albumin and globulin) were determined by using standard commercial kits (Biolabo, SA).

Histopathological examination

Livers of the female rats from different groups taken on day 15 were fixed in 10 % formal saline, dehydrated in ascending series of ethanol, cleared in xylol then embedded in paraffin wax. Sections of 6 microns thick were cut and mounted on clean glass slides. After being dried, sections were stained with haematoxylin and eosin (Pearse, 1972). Histopathological examinations were undertaken through light microscopy and photographs were made using an electronic camera microscope.

Statistical analysis

The difference between groups were calculated by using statistical program SPSS (11.0) using One-way ANOVA-test. Differences between data were compared by least significant difference $p< 0.05$**.** All data were expressed as Mean ± Standard deviation. All statistical tests were done by using statistical program SPSS (11.0) (Snedecore & Cochran, 1971).

Results

Total Blood Serum proteins:

1-The results indicated a significant ($p<0.05$) decrease in mean of total protein of serum blood for female rats treated with (NFX ,CPX) 5.19±0.438 g/100ml, 5.1±0.434 g/100ml respectively when compared with control 5.82±1.125 g/100ml, while there was no significant decrease in total protein of ENX group 5.3±0.374 g/100ml when compared with control group.

2-Also the results showed a significant ($p<0.05$) decrease of globulin of NFX, CPX and ENX treated groups1.47±0.447 g/100ml, 1.38±0.461 g/100ml and 1.37±0.561 g/100ml respectively as compared to control group, albumin increased non significant in all treated groups compared to control group. Table (1).

Treated groups	Total Serum Protein (g/100ml) Mean ±SD	Albumin (g/100ml) Mean ± SD	Globulin (g/100ml) Mean ± SD
Control	5.82±1.125	3.45±0.445	2.37±0.766
NFX	5.19±0.438*	3.72±0.293	1.47±0.447*
CPX	5.1±0.434*	3.72±0.239	1.38±0.461*
ENX	5.3±0.374	3.93±0.444	1.37±0.561*

*$p< 0.05$ refers to a significant difference compared with control group.Number of animals for each group = 10 animal

Histological finding

Sections of the control pregnant rat livers, showed normal central veins (C.V.) and blood vein (B.V) (Fig.1). While liver sections in treated pregnant rats showing dilation of blood veins (D.B.V) (Fig.2), (Fig.3) and (Fig.4). Liver sections of control group showed the normal hepatocytes(H.) are arranged in strands around the central veins (C.V.), the liver strands are separated from each other by blood sinusoids (B.S), the hepatic cells (H.C) contain one or two spherical nuclei, and the cytoplasm is slightly eosinophilic (Fig. 5). Histological examination of liver sections from female rats treated with (NFX, CPX and ENX) from 1st day to 15th day of gestation, showed congestion of portal veins (C.P.V.), dilated of sinusoids spaces (D.B.S.), degenerated hepatocytes (D.), and necrotic nuclei (N.) (Fig. 6, 7, 8).

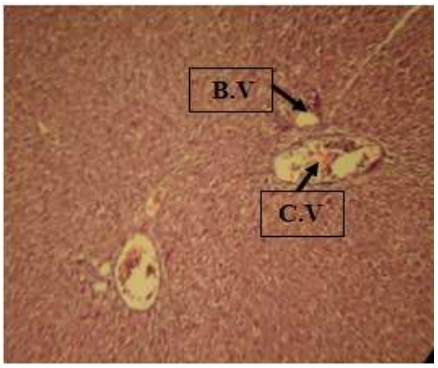

Fig.1: liver section of female in control group

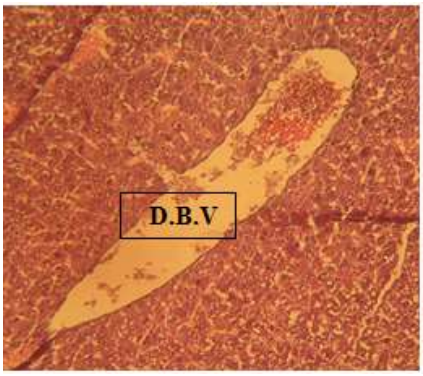

Fig.2: liver section of female in NFX group

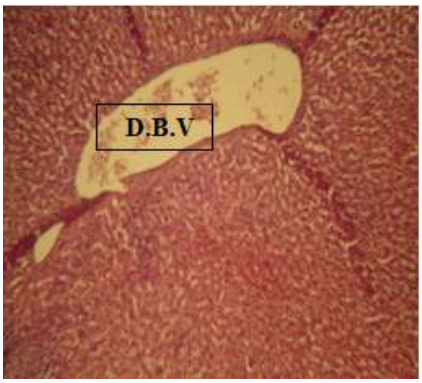

Fig.3: liver section of female in CPX group

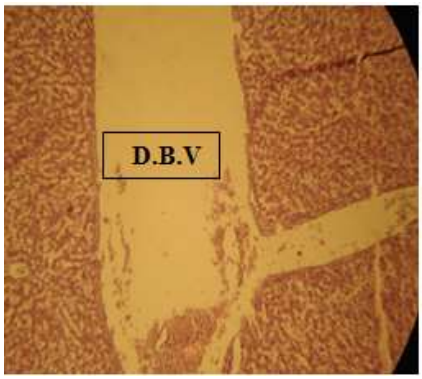

Fig.4: liver section of female in ENX group

Fig.1: Liver section at day 15 of gestation from control pregnant rats, showing normal central veins (C.V) and blood vein (B.V.), while (Fig.2), (Fig.3) and (Fig.4), showing dilation of blood veins (D.B.V) in Liver sections of NFX, CPX and ENX respectively treated pregnant rats. 10X H&E.

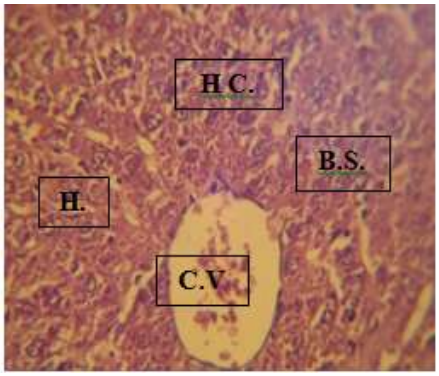

Fig.5: liver section of female in control group

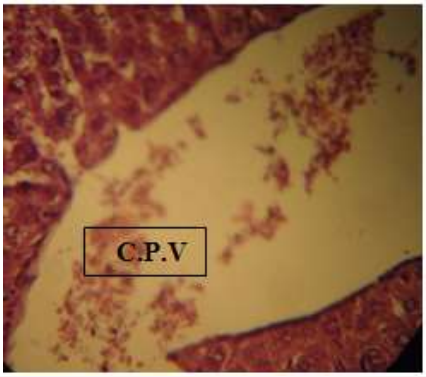

Fig.6: liver section of female in NFX group

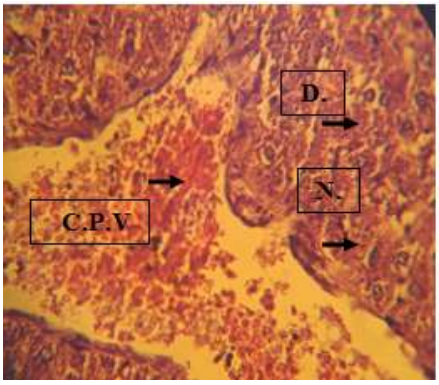

Fig.7: liver section of female in control group

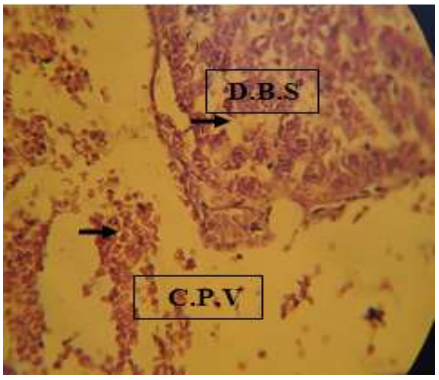

Fig.8: liver section of female in NFX group

Fig. 5: Liver section of control rats showing normal central vein (C.V.), hepatocyte (H.), hepatic cell (H.C.) and blood sinusoidal (B.S.). (Fig.6), (Fig.7) and (Fig.8) showing congestion of portal veins (C.P.V.) surrounded by inflammatory leucocytes infiltration, dilated of sinusoids spaces (D.B.S.), degenerated hepatocytes (D.), and necrotic nuclei (N.) in treated groups. 40X H&E.

Discussion

The results revealed a marked decrease in protein contents of livers of quinolones treated pregnant rats. Such reduction was dose and time dependant. It may be that the necrosed cells present in liver tissues and the marked infiltration of inflammatory cells are associated with drastic decrease in the protein content. This finding is in agreement with Minuk *et al.* (1997) who found that the quinolone antibiotic inhibits protein synthesis by interfering with DNA and RNA replication. Gilfillan *et al.* (1984) and Maura and Pino (1988) reported that the DNA damaging effect of norfloxacin in liver and kidney may be due to the fact that these organs play a major role in the metabolism and excretion of quinolones, the authors observed the concentrations of norfloxacin was higher in these organs than in serum and other organs. Maura and pino (1988) and Hanafy (2000) came to the conclusion that protein depletion is a consequence of nucleic acid diminution. It may be concluded that depletion of protein content in hepatocytes is a consequence of nucleic acids diminution and that mean there is a defect in DNA which leading to reduction of the synthesized protein. The studies dealing with quinolones toxicity on different body organs have established many histological alterations. On the other hand, contradictory results have reported the safety of the new quinolones in pregnancy. For instance, Kelly *et al.* (1998) found that CPX administration at a dose of 100 mg/kg, improved survival rates and hepatic regenerative activity in a rat model of fulminates hepatic failure. Minuk *et al.* (1995) and Zhang *et al.* (1996) reported that CPFX reverses the inhibitory effects in ethanol and carbon tetrachloride induced models of hepatic injury. The results obtained from the present study showed that administration of the flouruquinolones induced various changes in liver of pregnant rats. These changes varied from dilatation of hepatic portal vein and sinusoids, degenerative alterations degeneration progressed to necrosis. The quinolones are very important antimicrobials because they cover a wide variety of aerobic organisms. Although they are generally considered nontoxic (Christ and Lehnert, 1990). Han *et al.* (1995) found that ciprofloxacin which is a fluorinated quinolone antibiotic, exerts relatively low occurrence of adverse side effects. This is due to the association between quinolones and histopathological changes reported in liver and kidney of pregnant rats. Choi *et al.* (1997) reported that rufloxacin had potent therapeutic effects, and stimulated the immune system. It seems possible that histopathological changes in liver of pregnant rats reported in the present investigation is due to the toxicity quinolones. Giamarellou *et al.* (1989) found that the maternal serum levels of ciprofloxacin are several times lower than those in non-pregnantwomen.Ciprofloxacin, pefloxacin and ofloxacin penetrated the placenta adequately and are

concentrated in the amniotic fluid (Montan *et al.*, 1984; Bergen *et al.*, 1985). Liver damage was previously observed by many authors, following quinolones treatment (Contreras *et al.*, 2001; Bataille *et al.*, 2002; Goetz *et al.*, 2003 and Zimpfer *et al.*, 2004), Such cases revealed extensive hepatocellular necrosis and mixed inflammatory infiltrate in livers of patients. The pathomechanisms of quinolones -related liver injury are still unclear as reported by Zimpfer *et al.* (2004). The formation of free radicals by quinolones in the microsomal system might provide an explanation to the mechanisms of adverse effects observed after administration of these drugs. The mechanism of radical formation by quinolones might be a result of metabolizing these drugs by cytochrome P450 and/or redox reaction. Xie *et al.* (2003) 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 quinolones suppresses relevant cytochromes P450 at the transcription level. The histochemical alterations observed in the present study were in parallel with the histopatholagical findings and added a great deal to its authenticity.

Conclusion

High doses of fluoroquinolones caused clear biochemical and histopathological changes in livers of pregnant rats, and because these drugs are mainly used now for the treatment of many diseases especially UTI so these drugs should be used under careful clinical supervision, especially during pregnancy.

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