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ASSESSMENT OF HISTOPATHOLOGICAL AND HISTOCHEMICAL CHANGES IN LIVER OF PREGNANT FEMALE RATS AND THEIR FETUSES FOLLOWING CIPROFLOXACIN ADMINISTRATION

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ABSTRACT

The present study was carried out on female albino rats weighing 130-160 g, through two periods of gestation (day 1 or day 6 up to day 19 of pregnancy) representing four experimental groups and one control group. Experimental animals were given daily oral dose (57 mg/Kg b.w., therapeutic dose) or 114 mg/K b.w. (double therapeutic dose) of ciprofloxacin (CPFX). Dissection was performed on day 20 of pregnancy. Histological changes in liver sections of pregnant rats were in the form of dilatation of central and portal vein, and sinusoidal spaces; appearance of macrophages and Kupffer cells in sinusoidal spaces and congestion in blood vessels, The hepatocytes showed cytoplasmic vacuolation and nuclear unrest, Inflammatory leucocytic infiltration and focal fibrosis were also observed.

Liver sections of fetuses obtained from pregnant rats treated with the (CPFX) revealed histopathological alterations similar to their mothers. Total proteins content decreased by -5.59% in GI, -8.60% in GIII and -6.83% in GIV. Group two (GII) recorded a lower level in total protein (-0.62%). Moreover a significant decrease in total protein of fetuses was manifested being 17.96%, 13.28%, 22.62% and 21.87% for the four groups, respectively. The hepatocytes of mothers exhibited a significant decrease in DNA content presenting 9.42%, 3.46%, 10.40% and 7.51, % in the four groups respectively. In the hepatocytes of their fetuses the DNA content recorded a significant decrease of 10.43%, 9.34%, 18.68% and 17.58% for the four groups respectively. These results emphasize the toxicity of CPFX and its teratogenic effects.

Key Words: Histopathological – Histochemical – Ciprofloxacin - DNA.

INTRODUCTION

Fluoroquinolones (FQS), such as ciprofloxacin (CPFX), represent an important class of antimicrobial agents used in treatment of a wide range of infectious diseasesin different organs such as urinary tract, bone and joint, lower respiratory tract and skin (Lietman, 1995). Ciprofloxacin is very active against wide variety of pathogenic bacteria including some gram-positive and most gram- negative organisms (Hooper and Wolfson, 1985).

Little is known about the toxic effects of ciprofloxacin on pregnant female rats. There has been a number of developmental toxicity studies showing maternal toxicity by fluoroquinolones, e.g. decreased body weight and reduced food intake in rats and rabbits (Kim *et al.*, 2000; Guzman *et al.*, 2003).

Chann and Janjua (2003) reported that ciprofloxacin administration during gestation caused severe liver damage; pyknotic nuclei within hepatocytes and few distinctly visible nucleoli in hepatocytes of Wistar albino rats.

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; Batailte *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 eucaryotic DNA topoisomerase III resulting in the suppression of DNA synthesis. Several quinolone antibiotics, including ciproflaxacin were assayed in the in *vitro* hepatocyte primary culture/DNA repair test. MeQueen 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 polymerase-primase 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. Earlier studies by Maura and Pino (1988) indicated 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 (Goral *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.

The present study was done to evaluate the effects of ciprofloxacin administration during gestation on histological and histochemical profiles of liver in pregnant rats and their fetuses.

MATERIALS AND METHODS

Thirty six adult virgin female rats weighing (130-160 g) were obtained from the Egyptian Organization for Vaccine and Biological Preparations at Helwan. After 2 weeks period of acclimatization, the females were placed in cages overnight with untreated males (1 male to 3 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 0. Pregnant females were weighed, housed six per cage and maintained under conditions of temperature and humidity on a 12:12 light/dark cycle. Water and food were available *ad libitum*. Pregnant female rats were arranged into 5 groups: the first group represented the control and received saline, and the other 4 groups received oral CPFX by gastric intubation. According to Table (1). The daily doses given were 57 mg/kg and 114 mg/kg. The administered doses for experimental animals were calculated according to Paget and Barnes (1964) conversion tables. On day 20 of pregnancy, the control and treated females were sacrificed under anaesthesia.

Histopathological examination:

Livers of the pregnant female rats and their fetuses from different groups taken on day 20 of gestation were fixed in 10 % formol 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.For histochemical study, total protein was estimated by using bromphenol blue technique (Mazia *et al.*, 1953) and Feulgen method was used for DNA demonstration (Pearse, 1972). The histochemical study was done using computer image analyzing system (Leica Model). Estimation of the optical density of ten cells in each group was made.

Table (1): Groups according to duration and doses.

Groups	Starting day	Ending day	The dose used
Control	1	19	Saline solution
I	1	19	Therapeutic (57mg/kg/day)
II	6	19	Therapeutic (57mg/kg/day)
III	1	19	Double therapeutic (114mg/kg/day)
IV	6	19	Double therapeutic (114mg/kg/day)

RESULTS

Histological finding:

Sections of the control female rat livers, showed that the hepatocytes 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 contain one or two spherical nuclei, and the cytoplasm is slightly eosinophilic (Fig. 1). Histological examination of liver sections from female rats treated with (CPFX) from 1st day up to 19th day of gestation group I (GI), showed dilated blood veins (D.B.V.); some inflammatory cells (I.C.) (Fig. 2); appearance of hepatic sinusoids as dilated irregular spaces (D.B.S.); presence of macrophages (M.) Kupffer cells (K.) and degenerative appearance within hepatocytes (Fig. 3). As (CPFX) was administered from 6th day to 19th day of gestation group II (GII) liver sections exhibited congestion in blood vessels (C.B.V) that were surrounded by inflammatory leucocytic infltration. Some of the nuclei manifested signs of condensated nuclear material verifying frank progressed pyknosis (P) (Fig. 4).

Alterations observed in hepatic sections obtained from pregnant rats given double the therapeutic dose of (CPFX) from day 1 to day 19 of gestation, group III (GIII), showed pathological responses in the nuclei of liver cells varying from karyolysis to almost complete necrosis, and presence of Kupffer cells (Fig. 5). Clear histopathological changes were identified in liver section obtained from pregnant rats

administered double dose of (CPFX) from day 6 to day 19 of gestation group 4 (GIV) that displayed different injuries. These were in the form of local fibrosos with few mononuclear leucocytic inflammatory cell infiltrations with neutrophils whereas some nuclei appeared with necrosis (Fig. 6).

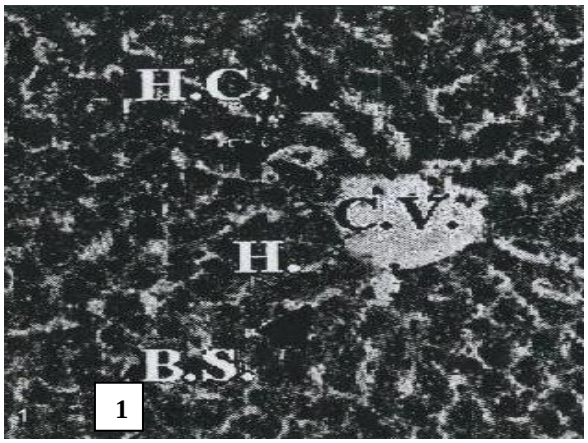
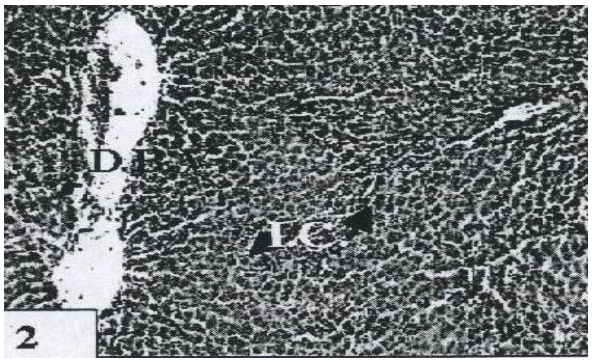
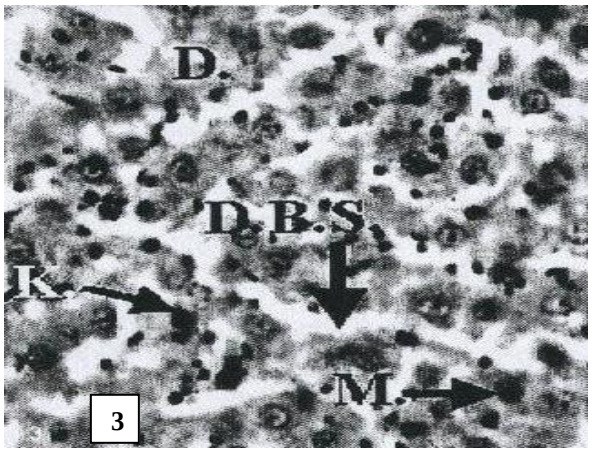


Fig. 1: Liver section of control pregnant rats showing central veins (C.V), hepatocyte (H.), hepatic cell (H.C.), blood sinusoidal (B.S.).





Figs. 2 & 3: Liver section from a pregnant rat treated with therapeutic dose CPFX for 19 days. Fig. 2 showed dilatation blood veins (D.B.V) some inflammatory cells (I.C.). Fig. 3 showed dilated of sinusoids spaces (D.B.S.), presence of macrophages (M), Kupffer cell (K) and degenarated hepatocytes (D).

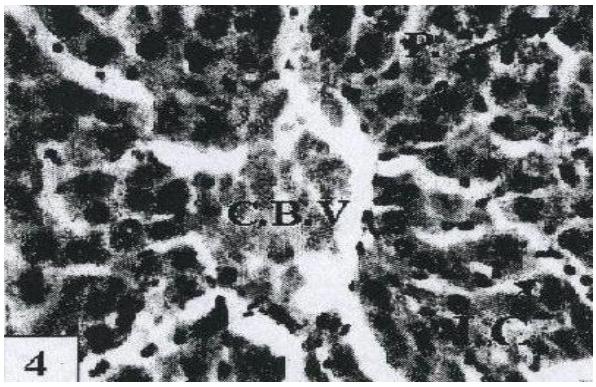


Fig. 4: Liver section from a pregnant rat treated with a therapeutic dose of CPFX for 13 days, showing congested blood vessels (C.B.V.), surrounded by inflammatory leucocytic infltration and pyknotic nuclei (P).

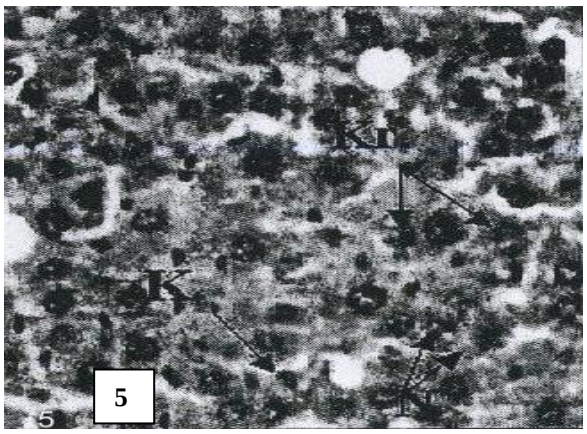


Fig. 5: Liver section from a pregnant rat treated with double therapeutic dose of CPFX for 19 days showing nuclei of liver cells with patholagical responses ranging from karyolysis (kr) to almost complete necrosis (N) and presence of Kupffer cells (K).

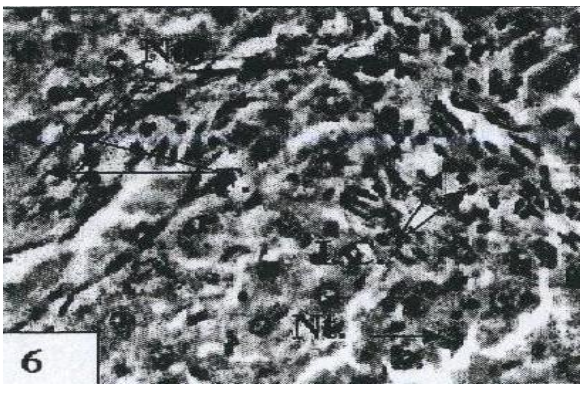


Fig. 6: Liver section from a pregnant rat treated with double therapeutic dose of CPFX for 13 days showing different injuries in the form of local fibrosos (F) with few mononuclear leucocytic inflammatory cells and neutrophils (Nt). Some nuclei appear suffering necrosis.

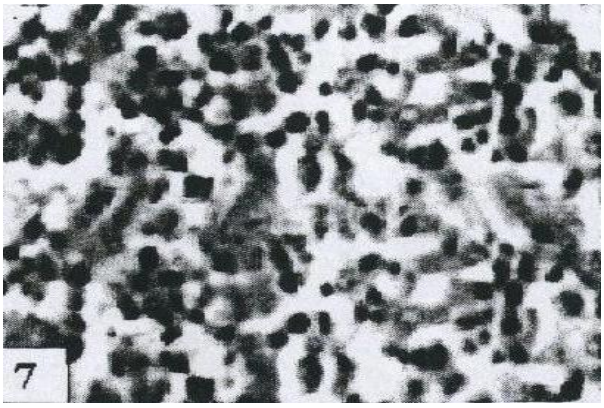
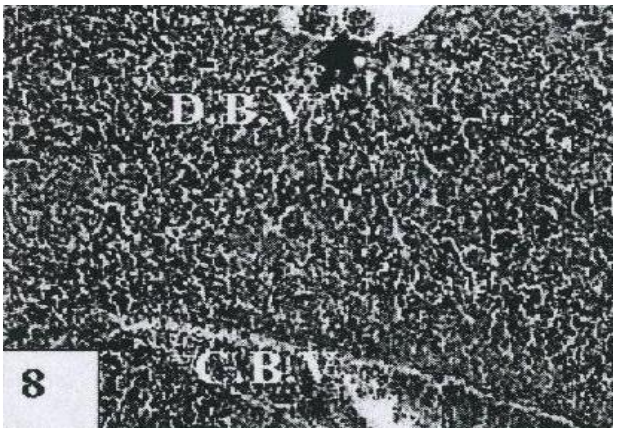
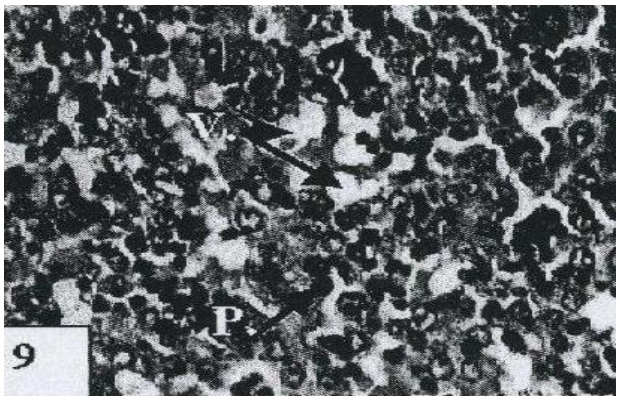


Fig. 7: Liver section of control fetus on day 20 of gestation, illustrating normal hepatic structure.

In liver sections of livers of fetuses on day 20 of gestation, normal hepatic structure was found (Fig. 7) The hepatic cells are large, polygonal in shape and possess coarsely granulated cytoplasm. They represent the different types of blood forming cells, namely the lymphocytes and erythroblasts. Liver sections of fetuses obtained from rats maternally receiving therapeutic doses of (CPFX) on day 1 up to day 19 of gestaion (GI), exhibited dilatation of central veins (D.B.V.) and congestion in portal veins (C.P.V.) (Fig. 8). Also some cells showed signs of pyknotic nuclei (P) and vacuolization of cytoplasm that might be attributed to lipolytic degeneration (V) (Fig. 9). Histopathological alterations in liver sections of fetuses obtained from rats treated with therapeutic doses of (CPFX) on day 6 up to day 19 of gestation, showed pathological responses in the nuclei of liver cells ranging from karyolysis to almost complete necrosis (Fig. 10). When (CPFX) was given in double therapeutic doses from day 1 up to day 19 of gestation (GIII), liver sections of fetuses obtained from these females, revealed congested portal veins (C.P.V.), with a considerable number of lymphocytes (L.) (Fig. 11). Fetal liver sections obtained from rats treated with double therapeutic dose from day 6 up to day 20 of gestation (GIV), manifested hepatocytes suffering from distinct lypolytic degeneration (D.) as reflected by their striking cytoplasmic vacuolizaion (V.), and necrotic nuclei (N.) (Fig. 12).





Figs. 8 & 9: Liver sections of fetuses obtained from pregnant rats receiving therapeutic doses of CPFX for 19 days (G1) showing dilatation of central veins (D.B.V.) and congestion of portal veins (C.B.V.) in (Fig.8). Also some cells showed signs of pyknotic nuciei (P.) and vacuolization of cytoplasm (V),(Fig. 9).

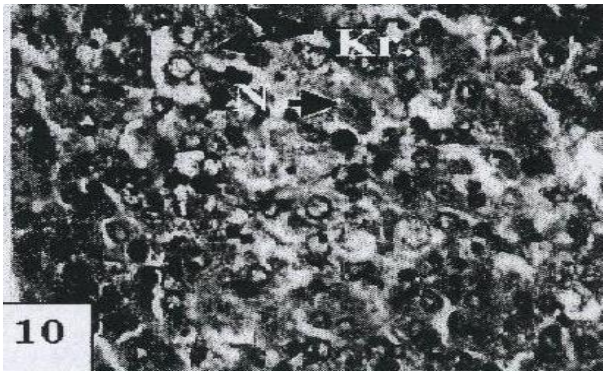


Fig. 10: Liver section of a fetus obtained from pregnant rats receiving therapeutic doses of CPFX for 13 days showing nuclei of some liver cells with pathological responses ranging from karyolysis (Kr) to almost complete necrosis (N).

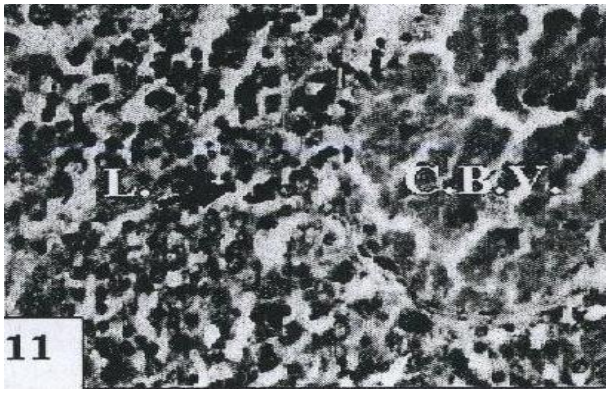


Fig. 11: Liver section of a fetus obtained from pregnant rats receiving double therapeutic doses of CPFX for 19 days (GIII) showing congestion in blood veins (C.B.V.), containing a considerable number of lymphocytes (L.).

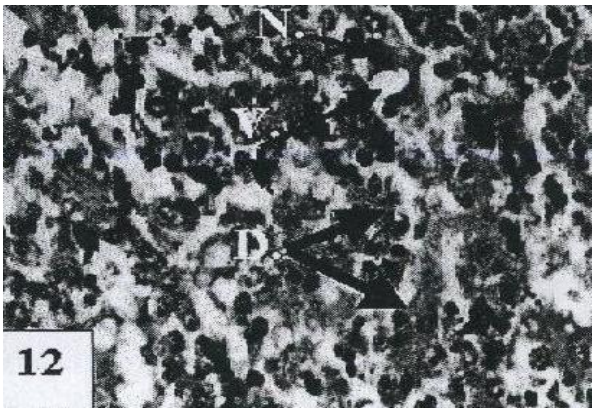


Fig. 12: liver section of a fetus obtained from pregnant rat receiving double therapeutic doses of CPFX for 13 days revealing hepatocytes apparently suffering from distinct lypolytic degeneration (D) and necrotic nuclei (N.).

Total protein content:

Normal distribution of total protein content in hepatocytes of control pregnant rats is given in (Fig. 13). The hepatocytes of pregnant rats administered (CPFX) for 19 days (GI) showed a gradual decrease in protein contents, (Fig. 14). In GII liver sections displayed the normal configuration with slight depletion of protein content (Fig. 15). After 19 & 13 days of treatment with double therapeutic doses of CPFX (G III & G IV), the total protein content decreased (Figs. 16 & 17). Liver sections obtained from fetuses belonging to previous expermintal groups showed a marked decrease in total protein content (Figs. 19-22) when compared with control (Fig. 18). Computer image analyzing (quantitatively) for total protein content in liver sections of mothers showed a significant decrease in treated groups by 5.59%, 8.60% and 6.83%, respectively, compared with the control; while GII which was treated with therapeutic doses from day 6 up to day 19 of gestation recorded a lower decrement by 0.62% when compared with control, (Table 2). The previous Table illustrated lower protein values in fetal liver sections; these values showed a significant decrease to 17.96%, 13.28%, 22.62% and 21.87% for the four experimental groups, respectively,as compared to the control.

Fig. 13: Normal distribution of protein content in the hepatocytes of control pregnant rat.

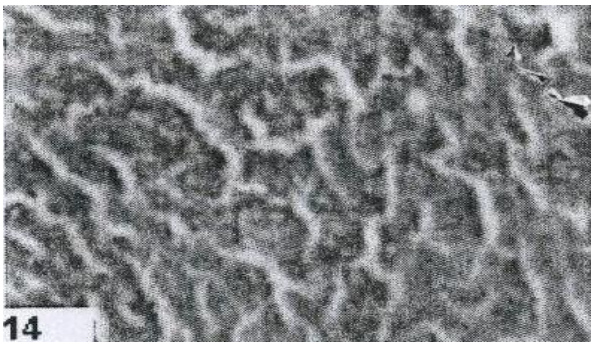


Fig. 14: Reduction of total protein content in the liver cells of a pregnant rat treated with therapeutic doses of CPFX on day 1 up to day 19 of gestation.

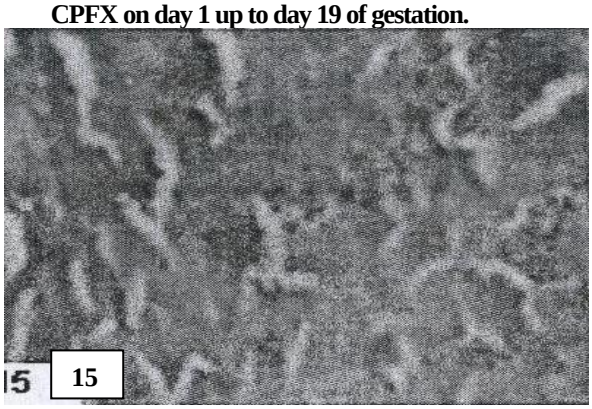


Fig. 15: Slight decrease of protein content in the liver cells of pregnant rats treated with therapeutic doses of CPFX on day 6 up to day 19 of gestation.

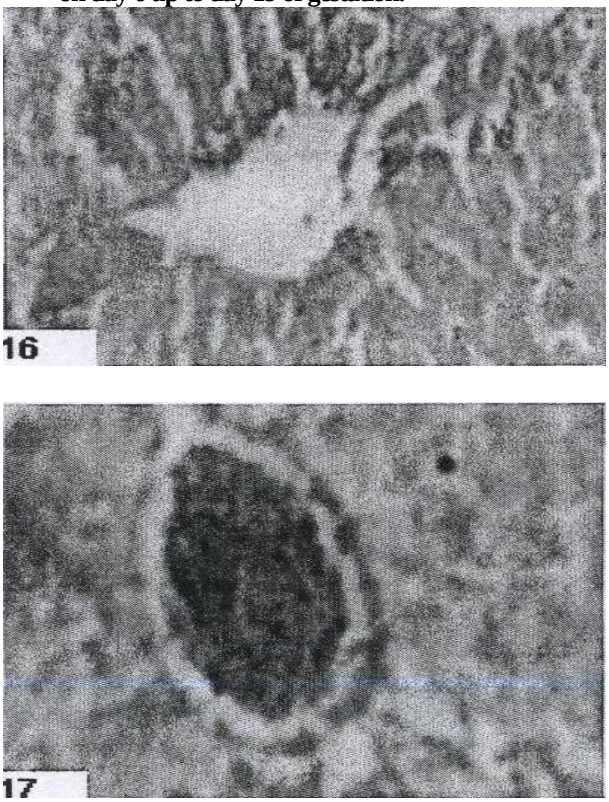


Fig. 16 & 17: Reduction of total protein content in the liver cells of rats treated with double therapeutic doses of CPFX for 19 days and 13 days.

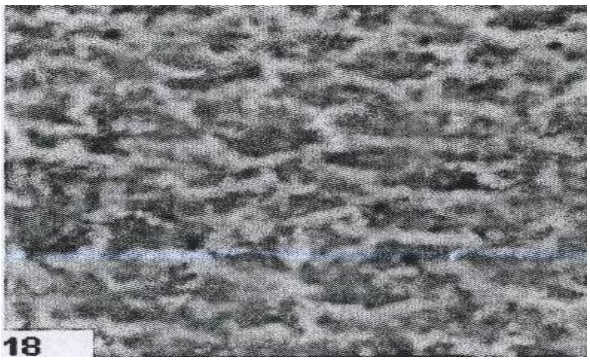
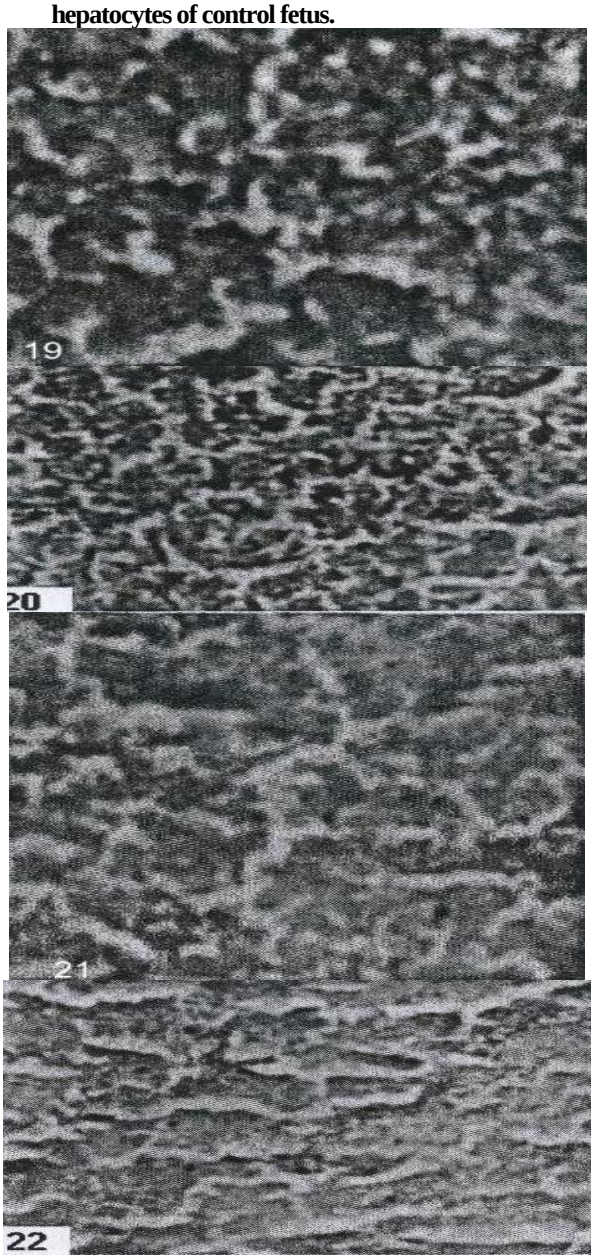


Fig. 18: Normal distribution of protein content in the hepatocytes of control fetus.



Figs. 19-22: Liver sections of fetuses belonging to previous experimental groups showing marked decrease in total protein content, when compared with control (Fig.18).

DNA

The DNA-containing (chromatin) particles in the normal hepatocytes were strongly stained red by using Feulgen method. These particles are distributed in the nucleoplasm (Fig. 23). The cytoplasm of these cells showed a negative staining. The nuclei of Kupffer cells were strongly stained. Liver sections examined after treatment with (CPFX) on day 1 up to day 19 of gestation (GI) showed a reduction in DNA (Fig. 24), whereas the same dose administered at day 6 up to day 19 of gestation (GII) showed few nuclei which were moderately stained (Fig. 25). Liver sections of rats treated with double the therapeutic doses of (CPFX) showed weak staining quality, (Figs. 26&27). Figure (28) shows the normal distribution of DNA content in liver tissue of control fetuses. Examination of liver sections of fetuses obtained after treatment with CPFX (GI) revealed a reduction in DNA (Fig. 29). GII showed a slight decrease in DNA content (Fig. 30). Fetuses obtained from pregnant rats treated with double therapeutic doses of CPFX revealed that most nuclei of the hepatocytes were weakly stained indicating more reduction for DNA (Figs 31 & 32).

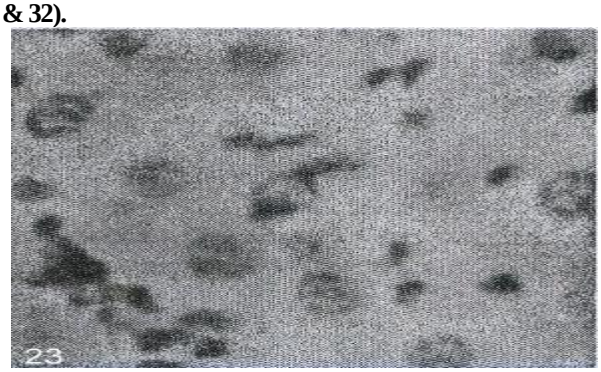


Fig. 23: DNA containing particles in the control liver cells were stained red colour with feulgen technique.

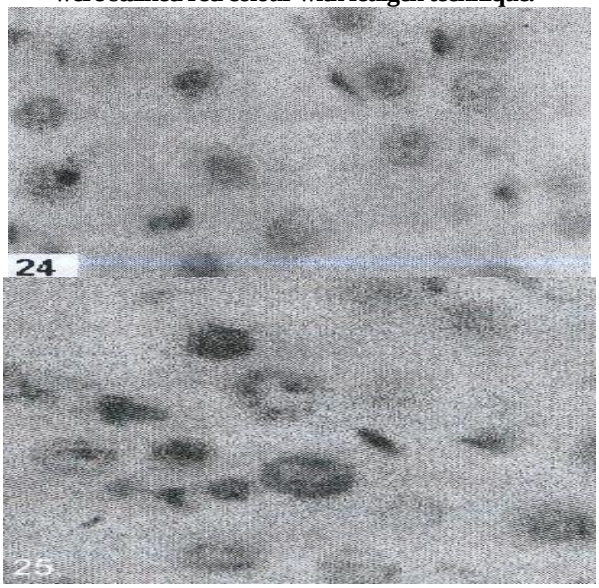
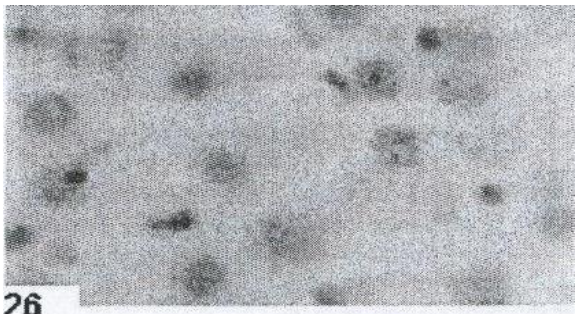
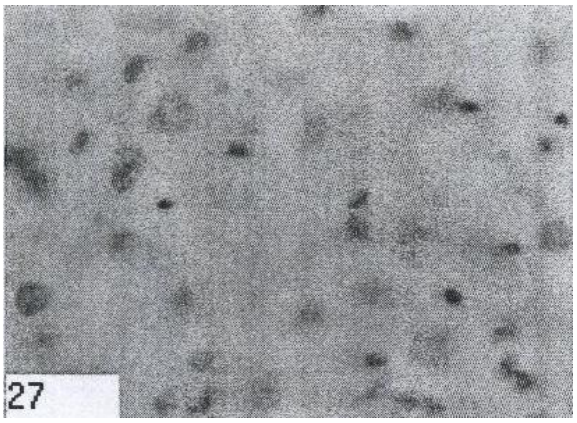


Fig. 24-25: Liver sections of rats treated with therapeutic doses of CPFX showing reduction in DNA. (Fig.24, GI) few nuclei were moderately stained (Fig. 25, GII).





Figs. 26-27: Liver sections of rats treated with double therapeutic doses of CPFX for 19 & 13 doses showing weak staining quality.

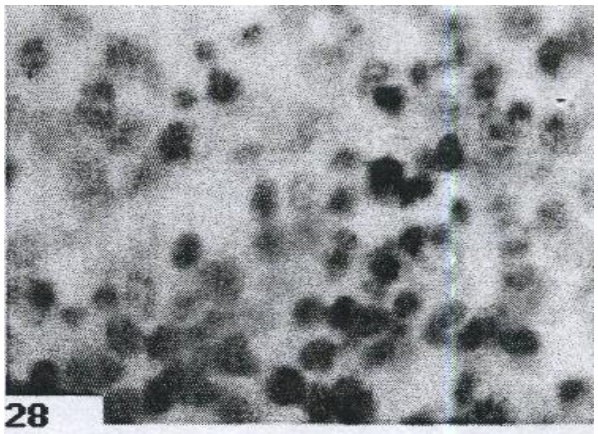
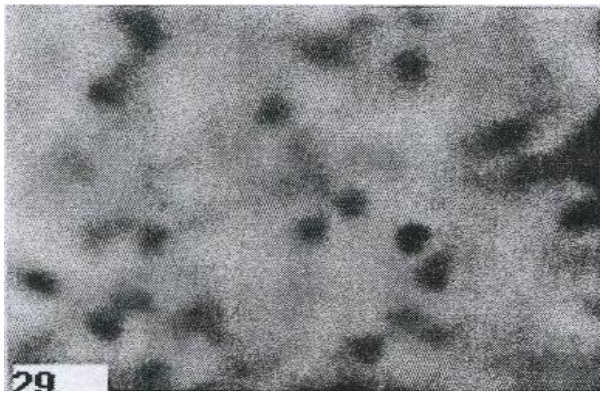
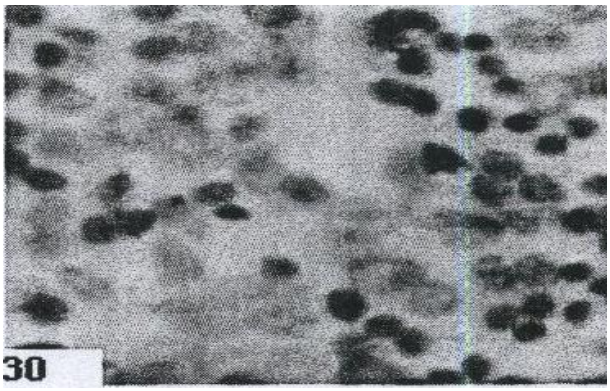
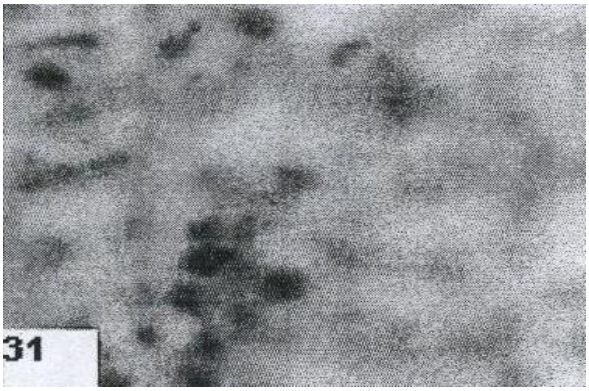


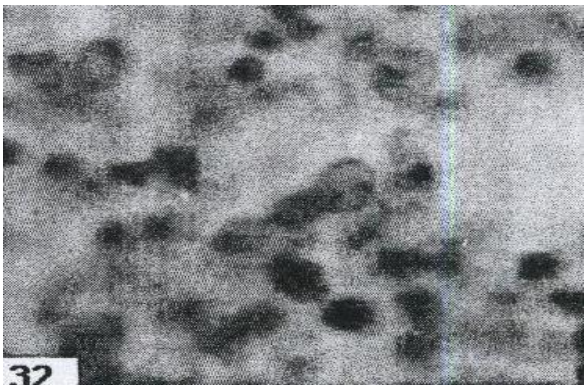
Fig. 28: Liver section of control fetus 20 days of gestation, showing normal distribution of DNA content.





Figs. 29-30: Liver sections of fetuses obtained from rats treated with therapeutic doses of CPFX for 19 & 13 days of gestation showed reduction in DNA (Fig.29, GI) and slight decrease in DNA content (Fig. 30, GII)





Figs. 31& 32: Liver sections of fetuses obtained from rats treated with double therapeutic doses of CPFX for 19&13 days of gestation demonstrating that most nuclei of the hepatocytes were weakly stained.

It is evident from the results in Table (1) and Fig. (33) that there was a significant decrease in DNA content after CPFX treatment at therapeutic as well as at double therapeutic doses (10.43%, 9.34%, 18.68% and 17.58%, respectively), in the 4 groups used. This decrease in DNA content in therapeutic doses was significant in GI ($p<0.01$), but nonsignificant in GII, ($p> 0.1$). On the other hand, the double therapeutic doses recorded a highly significant decrease in DNA content in GIII & GIV ($p<0.001$).

Table (2): Image analysis of total protein and DNA contents of livers of pregnant rats and their fetuses after CPFX administration.

Parameters		Group				
		G	G I	G II	G III	C IV
Total protein of mother	Mean±SD % of change P	1.61±0.09	1.52±0.1 -5.59 < 0.2	1.6± 0.09 -0.62 < 0.9	1.48*± 0.13 -8.6 < 0.02	1.5*± 0.78 -6.83 < 0.05
Total protein of Fetuses	Mean ±SD % change P	1.28±0.23	1.05±0.15 -17.96 < 0.1	1.11±0.19 -13.28 <0.2	0.99*±0.1 -22.62 <0.02	1* ±0.08 -21.87 <0.02
DNA content of mother	Mean ± SD % change P	1.73±0.077	1.57*±0.08 -9.24 < 0.02	1.67±0.06 -3.46 <0.2	1.55*±0.17 -10.40 < 0.01	1.6*±0.06 -7.51 <0.01
DNA contents of Fetuses	Mean ± SD % change P	1.82±0.11	1.63*±0.08 -10.43 <0.01	1.65±0.16 -9.34 >0.1	1.48**±0.05 -18.68 <0.001	1.5**±0.073 -17.58 <0.001

No. of animals = 6 for each group , *significant , **Highly significant , P probability value

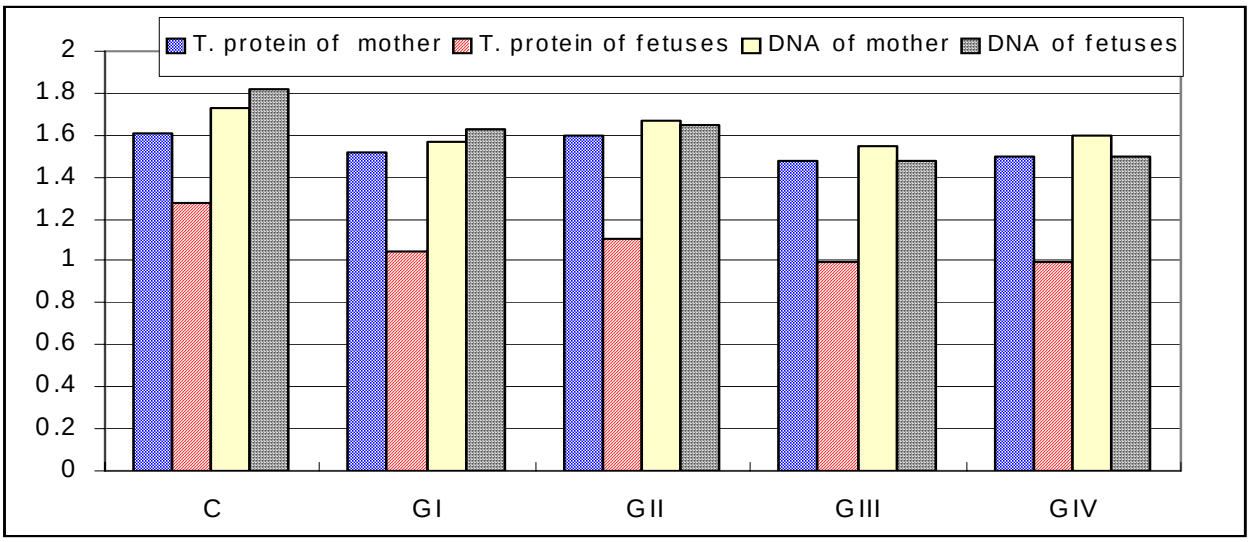


Fig. 33: Average of total protein & DNA content of control , treated rats and their fetuses.

Note: C= control, GI= group receiving therapeutic dose of CPFX from day 1 to day 19, GII= group receiving therapeutic dose of CPFX from day 6 to day 19, GIII= group receiving double therapeutic dose of CPFX from day 1 to day 19 and GIV= group receiving double therapeutic dose of CPFX from day 6 to day 19

DISCUSSION

The studies dealing with CPFX toxicity on different body organs have established many histological alterations in response to the therapeutic doses administered and to the period of administeration. On the other hand, contradictory results have reported the safety of the new quinolones in pregnancy. For instance, Kelly *et al.* (1998) found that CPFX administered 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 therapeutic and double therapeutic doses in two periods (preimplantation and postimplantation of pregnancy) induced various changes in liver of pregnant rats and their fetuses. These changes varied from dilatation of hepatic portal vein and sinusoids, increase in Kupffer and inflammatory cells, degenerative alterations and massive number of lymphoid cells aggregation in the portal area. Degeneration progressed to necrosis and pyknotic nuclei, and focal fibrosis. In addition, the liver of the fetuses showed fatty changes, haemolysis of the blood in sinusoidal spaces and severe dilatation of central vein with focal hemosiderosis, necrosis and pyknotic nuclei.

The quinolones are very important antimicrobials because they cover a wide variety of aerobic organisms. Although they are generally considered nontoxic (Christ *et al.*, 1988). 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 (CPFX) and histopathological changes reported in liver and kidney of pregnant rats and their fetuses. 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 and their fetuses reported in the present investigation is due to the maternal toxicity and developmental toxicity of CPFX. The present results are in agreement with Chernoff *et al.* (1989), who showed a relationship between maternal toxicity and developmental toxicity. Ledger (1977) reported that the changes in body compartments during pregnancy will influence the attainable serum levels of drugs. The auther added that the increase in maternal intravascular volume, the increased renal blood flow and the disposition in the fetal-placental may contribute to the lower serum levels of antibiotics in pregnant women.

Giamarellou *et al.* (1989) found that the maternal serum levels of ciprofloxacin are several times lower than those in non-pregnant women. 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 ciprofloxacin treatment (Contreras *et al.*, 2001; Batailtle *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 ciprofloxacin-related liver injury are still unclear as reported by Zimpfer *et al.* (2004). The formation of free radicals by (CPFX) in the microsomal system might provide an explanation to the mechanisms of adverse effects observed after administration of this drug. The mechanism of radical formation by CPFX might be a result of metabolizina this drug 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 ciprofloxacin 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. The results revealed a marked decrease in protein contont and DNA contents of livers of ciprofloxacine treated pregnant rats and their fetuses. 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 resplication.

Channa and Janjua (2003) atudied the effect of ciprofloxacin on feetal hepatocytes and found that, the number of hepatocytes showed a marked decrease per unit area while their size increased with decreased nuclear size which may be attributed to fat deposition and interference with RNA, DNA and protein synthesis in response to toxic effects of ciprofloxacin.

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 authers 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 the decline of DNA leading to reduction of the synthesized protein. Relevant features were reached by other investigators using different toxic agents (Elewa, 1995; El-Hady, 2000). These authors believed that irreversible damages, accentuated to necrotic areas, are due to a significant decrease in the number and degeneration in mitochondria, which are responsible for energy supply, and are due to drastic decrease in protein content resulting from their denaturation.

The highest dose in the present study produced a detectable amount of DNA damage in fetal tissues. This damage appears to be a specific consequence of maternal and fetal toxicity. Ciprofloxacine is commonly used for the treatment of various bacterrial infections. Its antibacterial activity has been ascribed to DNA binding, resulting in a marked inhilbtion of bacterial DNA topoisomerases (Gellert, 1981; Crumplin *et al.*, 1984; Gilman *et al.*, 1990; Abd-Allah *et al.*, 2000; Abdo llahi and Isazadeh, 2001; Kashidia *et al.*, 2002). Hussy *et al.* (1986) investigated the influence of 4-quinolones on mammalin topoisomerase II and eucaryotic DNA replication and reported that the order of potency of quinolones for inhibition of mammalian topoisomerase II was ciprofloxacin > norfloxacin > ofloxacin.

Nevertheless, other quinolones (ofloxacin) as well as other chemicals and drugs were also noticed by many researchers to exert the same depleting influence on the liver contents of protein and DNA as well as other organs (Abd-Allah *et al.*, 2000; Abdo llahi and Isazadeh, 2001; Kashida *et al.*, 2002). These authors reported that, ofloxacin induced marked disturbance in rat testicular DNA ploidy which may be explained on the basis of cross–reactivity to topoisomerase II.

The properties of fluoroquinolones that alter intracellular cAMP and calcium levels and their ability to suppress DNA, RNA and protein synthesis of acinar cells might be possible reasons for the observed changes. Inhibitory effects of flumequine (one of fluoroquinolones) on topoisomerare II were high relative to the influence on bacterial gyrase. The results of Kashida (2002) suggested that flumequine has initiating potential on mice liver that is atributable to induction of DNA strand breaks. These results confirm the findings in the presnt study.

El-Banhawy *et al.* (1992) described a marked depletion of protein content in the liver cells of rats after chloramphenicol. Nevertheless, it could be added in this regard that the primary target in such unusual circumstances is DNA since it is the template for RNA production leading to the protein synthesis. In other words the decline of DNA will result in a reduced amount of RNA leading eventually to a corresponding reduction of protein synthesis. These speculations receive marked support from the results achieved in the present investigation, where a marked loss in DNA has gone hand in hand with a corresponding decline of protein inclusion in the liver of mothers and their fetuses.

Conclusion

Both therapeutic and double therapeutic doses of ciprofloxacin caused clear histopathological and histochemical changes in livers of pregnant rats and their fetuses, so the drug should be used under careful clinical supervision, especially during pregnancy.

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