Animal Health Research Journal Vol. 6, No 3, September 2018 pp. 90-106

ISSN: 2356-7767

Comparative studies on amelioration of adverse effects of danofloxacin by using alpha-lipoic acid or ursodeoxycholic acid in rats

Hesham, S. Taha[*]; Riham, M. Raafat[*] and Dardiri M.[**]

*Biochemistry, Toxicology and Feed Deficiency Dept. (Pharmacology Unit) ,
**Pathology Depat, Animal Health Research Institute, Dokki, Giza, Egypt.

Received in 7/8/2018
Accepted in 16/9/2018

Abstract

Danofloxacin is a fluoroquinolone (FQ) antibacterial agent. The effect of danofloxacin on hepatic, renal tissues, blood biochemical parameters in rats and the effect of alpha lipoic acid (ALA) and ursodeoxycholic acid (UDCA) in ameliorating the likely deleterious effects were assessed in this study. Rats were divided into four groups (control, danofloxacin 30 mg/kg b.wt., danofloxacin 30 mg/kg b.wt.+ ALA 25 mg/kg b.wt., danofloxacin 30 mg/kg b.wt + UDCA 20mg/kg b.wt). Danofloxacin was injected intraperitoneally while ALA and UDCA was given orally. All treatments were given once daily for two weeks. Sera from all groups were collected and Liver functions (AST and ALT), kidney function (urea and creatinine), antioxidant parameters (glutathione (GSH) and lipid peroxidation marker (malondialdehyde (MDA) were measured in the sera of all groups. Post-mortem examination was performed on sacrificed rats and histopathological studies for liver and kidney were performed. Treatment of animals with danofloxacin caused significantly elevated activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine as well as MDA and decrease in GSH activity. Danofloxacin treated group showed histopathological changes such as diffuse vacuolar degeneration in the hepatocytes, severe vacuolar degeneration of the epithelial linings of some distal tubules with peritubular vascular dilatation and hemorrhage in kidney. All of these negative effects were significantly ameliorated by combination of ALA or UDCA with danofloxacin. Our findings suggest that *Salmonella* and *E. coli* organisms are still sensitive to danofloxacin with MIC_{90} of 0.049 µg/mL and 0.195 ug/ml respectively. It could be concluded that danofloxacin has adverse effect against liver and kidney function and these functions should be monitored, and the dose should be adjusted during danofloxacin therapy. Using of UDCA and ALA can reduce this negative effects which might be related to its antioxidant properties.

Keywords: *Danofloxacin, alpha-lipoic acid, ursodeoxycholic acid, rats.*

Introduction

Fluoroquinolones are most widely used today for the treatment of bacterial infections belongs to urinary tract or respiratory tract in human and animals. Whereas fluoroquinolones have been banned in some countries for use in poultry production due to development of FQ-resistant isolates, the use of these antimicrobials in poultry husbandry is still allowed in other countries with some restrictions. Resistance may develop by the micro-organisms being treated for example, through correct dosage or over prescription. The use of FQ in food producing animals has led to the emergence of FQ - resistant *Campylobacter*, *Salmonella* and *E. coli* with reduced susceptibility to fluoroquinolones with failure of human treatment. **(WHO, 1998)** .

Fluoroquinolones contain fluoride, which allows them to penetrate into sensitive tissues like brain and central nervous system, Fluoride

is a known neurotoxic agent (**Mandell and Tillotson, 2002)**. It carries severe side effects that can harm central nervous system, musculoskeletal, visual, renal system and most frequently linked to liver injury (**Hsiao *et al.,* 2010)** and nephrotoxicity **(Matsubara *et al.,* 2016).**

Ciprofloxacin, norfloxacin, levofloxacin, ofloxacin, trovafloxacin, enoxacin, sparfloxacin, grepafloxacin, gatifloxacin, clinafloxacin and moxifloxacin were conducted to reduce the incidence and features of fluoroquinolone nephrotoxicity, but the use of ciprofloxacin appears to increase the risk of the renal injury and this may be due to its longer and more widespread use **(Lomaestro, 2000).**

Danofloxacin is a synthetic fluoroquinolone antibacterial drug, developed for use in veterinary medicine. It has a rapid bactericidal activity against a broad range of pathogens responsible for a number of disease syndromes of economic importance in the commercial rearing livestock **(Norcia *et al.,* 1999**). The spectrum of activity of danofloxacin is wide and includes most gram-negative bacteria and some gram-positive bacteria, mycoplasmas, and intracellular pathogens, such as Brucella and Chlamydia species; but it has only limited activity against anaerobic organisms (**Hannan *et al.,* 1997**). It is administered in the drinking water to broiler chickens, calves, beef, cattle and non-lactating dairy cattle by intramuscular injection, for treatment of respiratory disease **(EMA, 1997).**

Alpha lipoic acid (ALA) contains two sulfhydryl groups which may exist in either oxidized or reduced states . The reduced form is called dihydrolipoic acid (DHLA) while oxidized form is usually referred as lipoic acid **(Nikolić *et al.,* 2014).** Lipoic acid (LA) in many tissues is rapidly converted to its redox couple, dihydrolipoic acid (DHLA). LA and DHLA have antioxidant properties are reflected by their direct ability to quench free radicals and indirect ability to recirculate cellular antioxidants (**Aly *et al*., 2009**). LA is a lipophilic antioxidant and an essential cofactor for mitochondrial respiratory enzymes. Initially, LA was tentatively regarded as a vitamin, found in spinach, broccoli, tomato, garden pea, rice bran and animal tissues **(Packer *et al* ., 2001).**

ALA act as a coenzyme in pyruvate dehydrogenase and alpha–ketoglutarate dehydrogenase mitochondrial reactions, leading to the production of cellular energy (ATP). It is well known that ALA and its reduced form, dihydrolipoic acid reduce oxidative stress by scavenging a number of free radicals in both membrane and aqueous domains by preventing membrane lipid peroxidation and protein damage through the redox regeneration of other antioxidants such as vitamins C and E, and by increasing intracellular glutathione (**Evans and Goldfine, 2000**).

ALA is a naturally occurring antioxidant and plays a fundamental role in metabolism. It has been shown that it affects the cellular processes, alters redox status of cells, and interacts with thiols and other antioxidants **(Packer *et al.,* 2001).**

It exhibited free radical scavenging activities and hepatoprotection properties against Carbon Tetrachloride-Induced Liver injury (**Mohamed *et al.,* 2014**) .

The liver damage induced by Acetaminophen was significantly ameliorated by α-lipoic acid and α-Tocopherol through amelioration of the mitochondrial oxidative stress in acetaminophen challenged rats (**Sudheesh *et al.,* 2013).**
Lipoic acid has antimicrobial activity against *Cronobacter sakazakii*, a gram-negative bacterium, cause bacteremia, necrotizing enterocolitis (NEC) and infant meningitis **(Nikolić *et al.,* 2014).**

Ursodeoxycholic acid (UDCA) is a naturally occurring tertiary dihydroxy hydrophilic bile acid used in the treatment of primary biliary cirrhosis. **(Poupon *et al.,* 1990)**. Food and drug administration approved UDCA for cholesterol gall stone dissolution, and primary biliary cir-

rhosis. UDCA is reported to increase bile flow, change the hydrophobicity index of the bile acid pool and has immune-suppressive effects. **(Bachrach and Hofmann, 1982)** .

UDCA a well-established therapy is used to treat liver dysfunction associated with various diseases, including cholestatic diseases and chronic active hepatitis **(Kotb, 2008)**. **El-Sherbiny *et al.* (2009)** found that ursodeoxycholic acid play a role in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats.

Ursodeoxycholic acid has effect against Helicobacter pylori with minimum inhibitory concentration of 400-800 µg /ml **(Itoh *et al.*, 1999).**

The beneficial effect of ursodeoxycholic acid in primary biliary cirrhosis is mediated in part by immunosuppression .It suppressed the production of IgM, IgG and IgA. Furthermore, ursodeoxycholic acid suppressed interleukin-2 and interleukin-4 production **(Yoshikawa *et al.*, 1992)**.

However, no information is available about the adverse effect of danofloxacin on liver and kidney and the role of lipoic acid or ursodeoxycholic acid in ameliorating this adverse effect if found. So, the present work has been designed to investigate the effect of danofloxacin on liver and kidney function and to evaluate the potential role of UDCA and ALA in prevention of hepatotoxic effect, nephrotoxicity and biochemical alterations that are induced by danofloxacin in rats**.**

Materials and Methods

Animals

Adult female rats weighing 250-350 g were used. Rats were kept in plastic cages and maintained at 22 ± 2^{o}C for one week before the start of the experiment. A standard diet and tap water were provided *ad libitum*.

Drugs and chemicals

Danocin (Danofloxacin 2.5%, injectable solution, was obtained from Adwia Co., Egypt).
Ursochol (Ursodeoxycholic acid (UDCA), 250 mg /capsule), was obtained from Medical Union Pharma (MUP), Egypt and was dissolved in 1% Tween 80 **(Khaled *et al.*, 2014)**.

Thiotacid (Alpha-lipoic acid, ALA), 300mg/ tablet) was obtained from Eva Pharma for Pharmaceuticals and Appliances and was dissolved in dimethyl sulphoxide (DMSO) **(Yasser *et al* ., 2015).**

Experimental design

Forty rats were randomly divided into four groups, each group consisting of ten rats each:

Group I: (Control group): Physiologic saline (0.09% NaCl) solution was administered intraperitoneal (I/P) in a single dose of 2.5 mg/ kg b.wt. for two weeks.
Group II: received danofloxacin (30 mg/kg b.wt.) by intraperitoneal (I/P) route once daily-for two weeks **(Dogan *et al.*, 2017**) .
Group III: received both Danofloxacin (30 mg/kg b.wt. IP) and ALA 25mg/kgb.wt., by oral gavage) once daily for two weeks
Group IV: received both Danofloxacin (30 mg/kg b.wt. IP) and UDCA (20mg/kg b.wt., by oral gavage) once daily for two weeks.
The experiment is designed according to **(Ihcène *et al.*, 2014 and Gamal *et al.*, 2009)**. There are no independent groups for ALA or UDCA where it has been proven that their results, whether histopathologIcal analysis or biochemical analysis, such as the control group **(Khaled *et al*., 2014 and Mohamed *et al.*, 2014**)

At the end of the experiment, blood samples were collected from the retro-orbital plexus and used for serum separation. Sera were used to determine ALT, AST, urea and creatinine levels. Immediately after blood collection, the animals were sacrificed by cervical dislocation. The liver and kidney of each rat was promptly removed and processed for histopathological

studies.

Assays to determine the antimicrobial activity (Agar well diffusion method)
MacConkey agar and salmonella shigella agar plates were inoculated with the bacterial suspensions (*E. coli* and *Salmonella typhimurium* respectively) using different concentration of danofloxacin (between 100 µg/mL and 0.049).

The plates were incubated at 37°C for 24 hours and the diameters of inhibition zones were measured using a caliper .Each assay was carried out in triplicate. **(Perez *et al.,* 1990).**

Determination of minimal inhibitory concentration (MIC) and, minimal bactericidal concentrations (MBC) against *E. coli* and *Salmonella*
MIC in MacConkey broth were determined by the broth dilution method according to CLSI **(Clinical and Laboratory Standards Institute, 2008)** against *E. coli* and *Salmonella* T. at concentrations between 100µg/mL and 0.049 µg/mL. For determination the MBC, all the tubes showing no bacterial growth in the MIC test were subculture. A standard loopful from each tube was inoculated on MacConkey agar and salmonella shigella agar plate. The plates were incubated at 35˚C for 18 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum **(Amita *et al.,* 2013).**

Biochemical Analysis
Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), urea and creatinine levels were deterimed using Spectrum kits **(Manufacturer: Spectrum Diagnostics/ Egypt)**. Malondialdehyde (MDA) was determined according to **(Ohkawa *et al*., 1979) and** glutathione (GSH) was assayed according to **(Tamura *et al.,* 1982)**.

Histopathological studies:
The animals were sacrificed by cervical dislocation at the end of the experiment specified time (two weeks) and specimens of livers and kidneys of each group were removed and fixed in 10% buffered neutral formalin solution . Tissue specimens were processed and embedded in paraffin, cut in sections of 4-5 nm thickness on microtome, stained with hematoxylin and eosin (H&E) and examined microscopically for the evaluation of histopathological changes **(Bancroft and Layton, 2012).**

Statistical analysis:
In this study, the values were expressed as mean ± standard deviation. The significance of the difference between each value presented by various groups was evaluated by one-way analysis of variance (ANOVA) and values with $P < 0.05$ were considered as statistically significant **(Kim, 2014)**.

Results and Discussion
The presence of Fluoroquinolones-resistant isolates should be routinely screened to preserve the efficacy of these important antimicrobial agents.
Antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans and animals. **Vanni *et al.* (2014)** reported that 24.2% of *E. coli* isolates were resistant to all tested Fluoroquinolones.

Therefore, Two methods are used to measure the activity of danofloxacin against the *E. coli* and Salmonella T., broth dilution method and agar dilution method. The bacteria are susceptible to danofloxacin with zones of inhibition ranging from 15.33 to 38.17 mm for *E. coli* and 11.33 to 37.68 mm for Salmonella T., so *E. coli* and *Salmonella T.* showed concentration-dependent susceptibility towards danofloxacin. The degree of susceptibility varied depends on the concentration of drug. **(tables 1 and 2)**.

The minimum inhibitory concentrations (MIC) of danofloxacin that prevented growth of 90% of *E. coli* and *Salmonella T.* were 0.195 µg/mL and 0.049 µg /ml and the MBC was 0.0.39 µg/

mL and 0.195 µg/ml respectively. The value may be close to that reported by **(Dirk** et al. **1992)** against *E. coli* (MIC_{90} was 0.25µg /ml) and against Salmonella (MIC with a value of 0.03 µg/ml, (**Xia *et al.,* 2018**).

European committee on antimicrobial susceptibility testing **(EUCAST)** currently provides only a susceptible category for Fluoroquinolone for Enterobacteriaceae with MIC ranged between ≤ 0.25 -1.0 µg/ml. **(EUCAST, 2017)**, Accordingly *E. coli* and *Salmonella* showed a high degree of susceptibility to danofloxacin.

Fluoroquinolones side effects include acute liver toxicity and acute kidney failure. However, an increasing number of evidence indicates that it has risk of elevation of the liver enzyme, cholestatic abnormalities and liver injury **(Carrascosa *et al.,* 2009 and Airey and Koller, 2003).**

The serum AST, ALT, urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of hepatic and renal dysfunction. **(Nnodim *et al.,* 2010).**

On microscopic examination of the liver and kidney tissues of the control animals group revealed a normal architecture **(Fig. 1 and $2_{a,b}$)** .

Data of serum samples obtained from rats received danofloxacin with or without of ursodeoxycholic acid or Alpha-lipoic acid were depicted in **(tables 3,4).**

In this study, we found significant elevation of serum ALT and AST, urea and creatinine activity in rats treated with danofloxacin which is similar to the results found in previous studies for Fluoroquinolones **(Wolfson and Hooper, 1991)**. Danofloxacin treated rats showed a significant increase in plasma AST, ALT, by 42.46% and 33.18%, respectively compared with control group and this percentage was decreased by addition of ALA to 14.02%, 24.42% and by addition of UDCA to 0.04% and 10.12%.

Similar results have been reported by (**Zulfiqar *et al.,* 2017)** who found that the Fluorinated quinolones, including ciprofloxacin, are metabolized in the liver and excreted by the kidneys and cause a 1% to 3% self-resolving transient elevation in liver enzymes in young and adult. Also Enrofloxacin caused an increase of ALT, AST and Alkaline Phosphatase (**Neer, 1988**). ciprofloxacin induces hepatotoxicity, cholestatic jaundice, elevated level of total bilirubin, alkaline phosphatase, aspartate aminotransferase and prolonged prothrombin time **(Hirch and Lundquist, 2009).**

Administration of different doses (5, 10, 20 mg/kg b.wt.) of levofloxacin significantly increased the plasma activities of ALT and AST by 37%, and 48% respectively, when compared to the control group (**Ebenezer *et al.,* 2015)** and this elevation due to necrosis of hepatocytes **(Pratt and Kaplan, 2000).** in addition, **TRASË *et al.* (2001)** found that Enrofloxacin caused a reversible increase in serum AST and indirect Bilirubin concentrations in dogs.

Urea is the first acute renal marker which increases when the kidney suffers any kind of injury. Otherwise, creatinine is the most trustable of them **(Borges *et al.* 2008).**

Our data showed a significant increase in plasma urea and creatinine levels in Group II by 44.39%, and 30.23% respectively compared with control group and then these percentages decreased to 14.31% and 13.95% in group III and decreased to 10.27% and 4.65% in group IV.

In previous study, oral ciprofloxacin therapy may lead to acute renal failure secondary to tubulointerstitial nephritis characterized by an increased creatinine to blood urea nitrogen (BUN) ratio **(Hootkins *et al.,* 1989).** Elevation of the plasma levels of creatinine and urea by

levofloxacin is an indication of abnormal renal function (**Mouton and Holder, 2006)**.

Our results demonstrated that administration of danofloxacin resulted in markedly significant decrease in the level of hepatic GSH compared to those of control group while GSH level significantly increased by addition of ALA or UDCA compared to those of danofloxacin treated group.

MDA levels in liver tissue (a marker of lipid peroxidation) was significantly increased in danofloxacin treated group (3.50 ± 0.40 nmol/ml) compared to those of control group (2.83 ± 0.37 nmol/ml) .

Similar observations were obtained by several authors following ciprofloxacin and levofloxacin treatment **(Rawi *et al.,* 2011**). **Elamaran *et al.* (2015)** reported that enrofloxacin induced oxidative stress in broiler**.**

Talla and Veerareddy (2011) reported that ciprofloxacin and levofloxacin induce more reactive oxygen species that lead to cell damage than gatifloxacin. The formation of free radicals by ciprofloxacin in the microsomal system might provide an explanation to the mechanisms of adverse effects observed after administration of this drug (**Xie *et al.,* 2003)**.

Lipoic acid is rapidly converted into its reduced form, dihydrolipoic acid (DHLA) . Recent studies demonstrated that LA and dihydrolipoic acid can act as potent antioxidants **(Ihcène *et al.,* 2014).**

A combination of danofloxacin with ALA causes significant improvement in liver function and significant reduction in liver enzyme activities (ALT and AST) than danofloxacin treated group. These results were in agreement with a report showing that pretreatment of rats with 100 mg/kg ALA, 1h before acetaminophen administration inhibited the elevation induced by acetaminophen in the activities of SGOT, SGPT and SAP and in the level of serum total bilirubin **(Ahmed *et al.,* 2008)**

Moreover, a significant decrease in urea and creatinine levels in danofloxacin + ALA compared to danofloxacin treated groups. These results came in accordance with the recorded data of **(Rashwan and Anfenan, 2012)** who, reported that, treatment with α-lipoic acid in cadmium intoxicated rats resulted in decrease in serum urea and creatinine concentrations compared to cadmium group.

The significant elevation in malondialdehyde (MDA) and lowering in GSH by danofloxacin was significantly ameliorated by combination of danofloxacin with ALA. **Elamaran *et al.* (2015**) found that treatment with alpha-lipoic acid (100 mg/kg b.w) significantly restored the antioxidant status in liver homogenates induced by Enrofloxacin in broilers .

ALA prevents cellular necrosis, this effect might be related to both its radical scavenging properties and indirect effect as a regulator of antioxidative systems. The protective role of ALA against hepatotoxicity and oxidative stress is well documented in a series of scientific reports **(Bae *et al.,* 2014)**.

Previous studies had reported that lipoic acid showed significant protective effects against tissue damage induced by some xenobiotics like arsenic (**Shila *et al.,* 2005)**, Bisphenol A (**El-Beshbishy *et al.,* 2012)** and Adriamycin **(Prahalathan *et al.,* 2006).**

Ursodeoxycholic acid (UDCA) is one of the secondary bile acids. Combination of danofloxacin plus UDCA causes significant reduction in liver enzyme activities such as AST and ALT (62.17 ± 2.25 U/L, 22.63 ± 0.50 U/L) compared to group III (67.98 ± 1.58 U/L , 25.57 ± 0.89 U/L) and Group II (84.94 ± 2.62 U/L , 27.37 ±1.51 U/L) respectively.

This injury induced finding was contrary to the results of previous study where in UDCA stabilizes the mitochondrial and plasma mem-

branes of hepatocytes that protect them from various other injuries and it constitute an antiapoptotic action (**Sola *et al* ., 2007**). This protective effect is probably due to its antioxidant action (**Lukivskaya *et al.,* 2006)** .

In addition, UDCA is an effective hepatoprotective agent against liver dysfunction caused by the broad spectrum antibiotic combination amoxicillin-clavulanic acid (**El-Sherbiny *et al.,* 2009)** and it protected mice from liver by isoniazid plus rifampicin (**Chen *et al* ., 2011).**

Khaled *et al.* (2014) found that UDCA has been reported to protect against ceftriaxone - induced liver injury in rats and normalize the elevated hepatic MDA, and nitric oxide (NO) induced by ceftriaxone and it restored GSH levels in rats. The normalization of serum markers by UDCA suggests that it is able to protect the membrane integrity against ceftriaxone that induces leakage of marker enzymes into the circulation.
In the present work, a significant decrease in the level of urea and creatinine was observed in Group IV (danofloxacin + UDCA) compared to group II. Similar results have been reported in mice, showed that UDCA alleviated blood urea nitrogen (BUN), serum creatinine (SCr) and exerts renoprotective effects **(Aili *et al.,* 2016).**

Addition of UDCA to danofloxacin lead to decrease the elevated biochemical oxidative stress markers; hepatic MDA (2.78 ± 0.36 nmol/ml) and it restored GSH levels (4.93± 0.96 ug/ml) compared to danofloxacin treatment group (3.50 ± 0.40 nmol/ml, 2.87 0.31 ug /ml respectively) .

This result is in agreement with that found in previous studies of UDCA that significantly increased the levels of GSH, thereby protecting hepatocytes against oxidative injury **(Mitsuyoshi *et al.,* 1999).**

El- Sherbiny *et al.* (2009) have reported that UDCA was able to normalize the elevated biochemical oxidative stress markers, reactive oxygen species (ROS) production and restored GSH normal level. They found that UDCA has a hepatoprotective effect on liver dysfunction caused by Co-amoxyclav and this effect is attributed to its antioxidant properties and this could be clinically beneficial to reduce the hepatotoxic adverse effect of Co-amoxyclav.
Histopathological examination of the liver and kidney tissues in this study confirmed the biochemical results.
The control group showed normal hepatic tissue architecture, normal glomeruli and normal renal tubules **(Fig. 1, $2_{a,b}$).**
The group treated with danofloxacin alone showed diffuse ballooning degeneration of hepatocytes and dilatation of central veins as well as desquamation of the epithelial lining of some proximal tubules and severe vacuolar degeneration of the epithelial linings of some distal tubules with peritubular vascular dilatation and interstitial hemorrhage **(Fig. 3,$4_{a,b}$).**

Similar observation by several authors following ciprofloxacin treatment were reported. **Nada (2012**), **AL- Rikaby *et al.* (2016)**, **Elbe *et al.* (2016**) **and Taslider *et al.* (2016**) who found that ciprofloxacin has the potential to induce hepatotoxicity and nephrotoxicity effects in rats. They reported that ciprofloxacin treatment caused vacuolation of hepatocytes, dilatation of central vein and hepatocellular necrosis ,while the kidney tissues displayed vacuolation of tubular epithelium of cortical areas, dilatation of tubules and coagulative necrosis of renal tubular epithelium .

Moreover, **Ravikumar *et al.* (2017)** found similar histopathological changes in levofloxacin treated birds induced nephrotoxicity. They observed tubular epithelial cell degeneration, desquamation, congestion, hemorrhages, and necrosis along with infiltration of inflammatory cells in the interstitum.
Formation of free radicals by ciprofloxacin in the microsomal system might provide an explanation to the mechanisms of adverse effects observed after administration of drug in rats.

The mechanism of radical formation by ciprofloxacin might be a result of metabolize this drug by cytochrome P450 and/or redox reaction **(Nadia, 2006). 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.

In our study, the group of animals treated with danofloxacin in combination with ALA, the liver showed a marked improvement of hepatocellular degeneration with restoration of liver tissue architecture and apparently normal hepatocytes with very mild ballooning degeneration still present in some few hepatocytes in comparison with the group treated with danofloxacin alone. The kidney tissue showed relatively restoration of normal appearance of epithelial lining of proximal convoluted tubules, apparently normal glomerulous and relatively normal distal convoluted tubules in group III **(Fig. 5,6)**

It has been found that ALA protects against gentamicin (**Sandhya and Varalaskshmi, 1997**), cisplatin (**Somani *et al.,* 2000**), chloroquine (**Murugavel and Pari, 2004**) and cyclosporine (**Amudha *et al.,* 2006**) induced nephrotoxicity in rats. Moreover, the protective role of ALA against hepatotoxicity and oxidative stress is well documented in a series of scientific reports (**Bae *et al.,* 2014**).

ALA produced its protective effect by preventing the increase in lipid peroxidation level and by increasing the inhibited activities of enzymatic antioxidants and decreased levels of non-enzymatic antioxidants **(Morakinyo ET AL., 2012).**

In the present work, liver from animals treated with danofloxacin plus UDCA showed also marked improvement of hepatic cell degeneration which restricted to few hepatocytes with mild ballooning degeneration. Moreover, the kidney tissues showed apparently normal proximal and distal tubules with presence of few necrotic tubular epithelial cells (the cells were swollen with deeply eosinophilic cytoplasm with karyopyknotic nuclei). (Fig. 7,8).

Our results agree with the findings of **Khaled *et al.,* 2014** who found that UDCA showed mild hydropic degeneration in the periportal (peripheral) area and normal hepatocytes in the central lobular area compared to ceftriaxone which showed nearly complete lobular degeneration and the effect of UDCA might be related to its antioxidant properties.

UDCA Stabilizes the mitochondrial and plasma membranes of hepatocytes that protect them from various other injuries and it constitute on antiapoptoic action **(Sola *et al.,* 2007**). **Abd-Elhamid *et al.* (2018)** found that UDCA ameliorates gentamicin--induced kidney injury via inhibition of oxidative stress, inflammation and apoptosis.

Conclusion

Our results demonstrated that danofloxacin which is a fluorinated quinolone antibiotic can cause adverse side effects such as hepatotoxicity and nephrotoxicity in rats. ALA and UDCA have an ameliorating effect against these adverse reactions which were evidenced by improvement in biochemical measurements and histopathological changes and so it is useful for reducing and/or preventing danofloxacin induced liver and kidney damage. Therefore , it is necessary to monitor liver and kidney functions when giving danofloxacin for long periods or for high doses, if necessary, these acids (ALA and UDCA) can be used to protect the above organs from the side effect of the drugs. Danofloxacin still have a good activity against *E. coli* and *Salmonella typhimurium.*

Table (1). Antimicrobial activity (zone of inhibition) of danofloxacin against *E. coli* and *Salmonella Typhimurium* by agar well diffusion method (Mean ± SD).

Danofloxacin Conc (ug/ml)	Zone of diameter of inhibition (mm)	
	E. coli	***Salmonella Typhimurium***
100	38.17 ± 0.28	37.68 ± 0.96
50	36 .67 ±0.57	34.33 ± 0.57
25	34.33± 0.58	32.33 ± 0.29
12.5	31.80± 0.29	28.67 ± 0.29
6.25	0 28.50± 0.5	26 .33 ± 0.57
3.125	26.16± 0.28	24.16 ± 0.28
1.563	21.9± 0.11	22.40 ± 0.69
0.781	19.10 ± 0.36	19.46 ± 0.42
0.39	17.17 ± 0.29	16.9 ± 0.36
0.195	15.33± 0..28	15.43 ± 0.37
0.097	ND	13.53 ± 0.46
0.049	ND	11.33 ± 0.58

ND : not detected .

Table (2). Minimum inhibitory conc. (MIC) and Minimum bactericidal conc.(MBCs) (ug / ml) for danofloxacin against *E.coli* and *Salmonella Typhimurium.*

Organism	MIC	MBCs
E. coli	0.195	0.39
Salmonella Typhimurium	0.049	0.195

Table (3). Effect of danofloxacin (30 mg/kg b.wt.) with or without of ursodeoxycholic acid (20mg/kg b.wt) or Alpha-lipoic acid (25mg/kg b.wt) on some serum biochemical parametersin rats (mean ± SD).

Parameters / Groups	AST (U/L)	ALT (U/L)	Urea mg/dl	creatinine mg/dl
Group I (Control)	59.62 ± 2.82	20.55 ± 0.87	22.57 ± 0.98	0.43 ± 0.06
Group II (danofloxacin)	84.94 ± 2.62 [a] **(42.46%)[d]**	27.37 ± 1.51 [a] **(33.18 %) [d]**	32.59 ± 0.45 [a] **(44.39 %) [d]**	0.56 ± 0.09[a] **(30.23%) [d]**
Group III (Danofloxacin+ ALA)	67.98 ± 1.58 [ab] **(14.02 %) [d]**	25.57 ± 0.89 [ab] **(24.42%)[d]**	25.80 ± 1.09 [ab] **(14.31%)[d]**	0.49 ± 0.11 **(13.95%) [d]**
Group IV (Danofloxacin + UDCA)	62.17 ± 2.25 [bc] **(0.04 %)[d]**	22.63 ± 0.50 [abc] **(10.12%)[d]**	24.89 ± 1.18[ab] **(10.27%)[d]**	0.45 ± 0.07[b] **(4.65%) [d]**

[a]significantly different from the control group at p< 0.05
[b] significantly different from danofloxacin group at p< 0.05
[c] significantly different from Danofloxacin+ ALA group at p< 0.05
[d] The percentage increase compared to the control group.

Table (4). Mean values of antioxidive and lipid peroxidation marker of rats given danofloxacin (30 mg/kg b.wt.) with or without of ursodeoxycholic acid (20mg/kg b.wt) or Alpha-lipoic acid (25mg/kg b.wt.) (mean ± SD)..

Groups / Parameters	GSH ug/ml	MDA nmol/ml
Group I (Control)	3.86 ± 0.67	2.83±0.37
Group II (danofloxacin)	2.87± 0.31 [a] 25.64 **%** [e]	3.50±0.40[a] 23.67**%** [d]
Group III (Danofloxacin+ ALA)	4.79±0.61 [b] 24.09**%** [d]	2.79±0.33[b] 1.41**%** [e]
Group IV (Danofloxacin + UDCA)	4.93±0.96 [a b] 27.72**%** [d]	2.78±0.36[b] 1.76**%**[e]

[a] significantly different from the control group at p< 0.05
[b] significantly different from danofloxacin group at p< 0.05
[c] significantly different from Danofloxacin+ ALA group at p< 0.05
[d] The percentage increase compared to the control group.

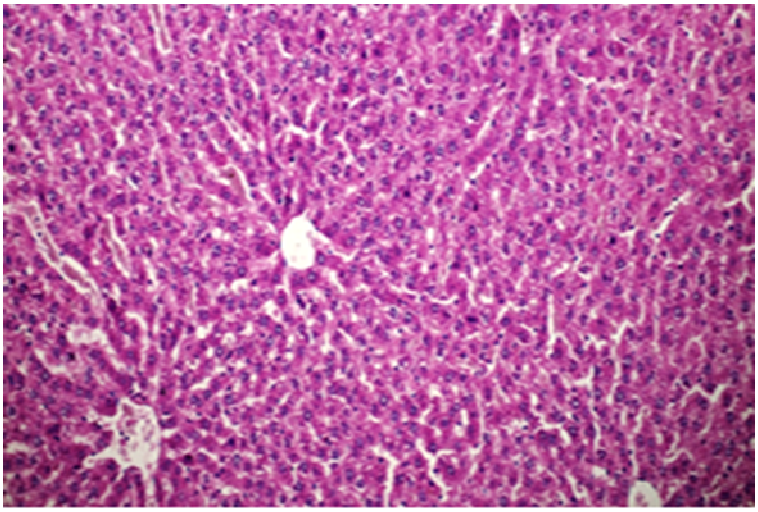

Figure (1). Liver of a rat of control group showing the normal hepatic tissue architecture with normal hepatocytes (H&E, X 200)

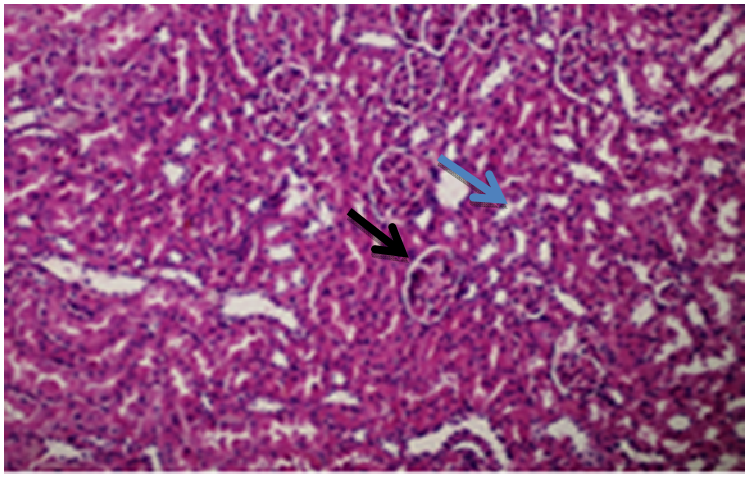

Figure (2a). Kidney of a rat of control group showing normal structure of glomeruli (black arrow), normal proximal convoluted tubules (blue arrow) (H&E, X 200)

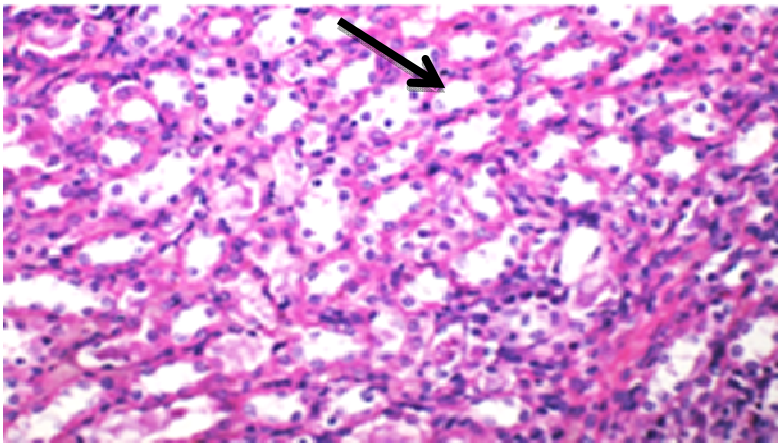

Figure (2b) Kidney of a rat of control group showing normal distal renal tubules (arrow), (H&E, X 400)

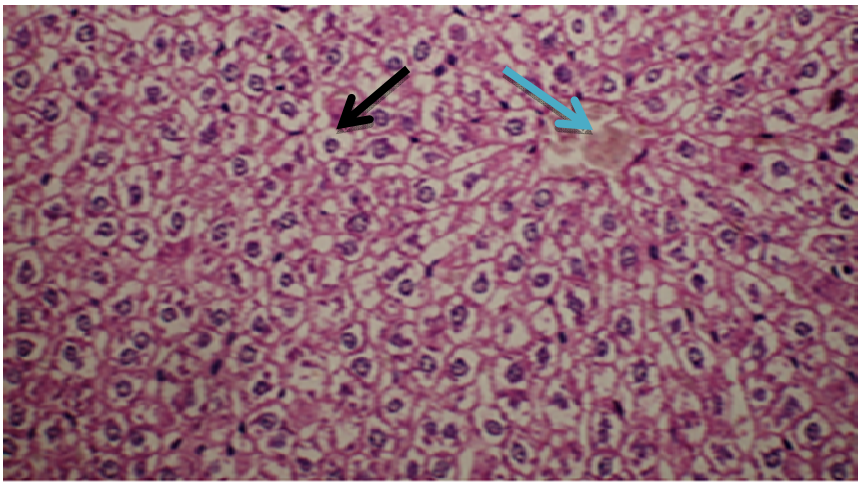

Figure (3). liver of a rat treated with danofloxacin showing diffuse ballooning degeneration of hepatocytes (black arrow) together with dilated blood vessel (blue arrow) (H&E, X 400)

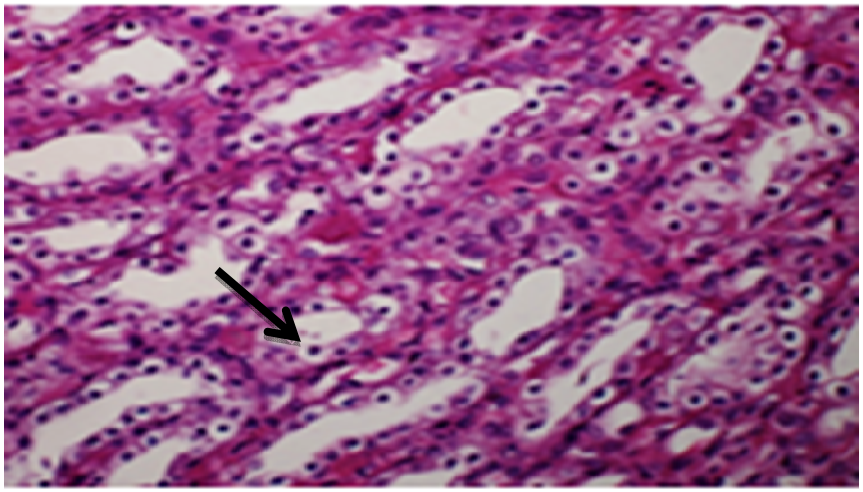

Figure (4a). kidney of a rat treated with danofloxacin showing vacuolar degeneration of the epithelial linings of distal tubules (arrow) (H&E, X 400)

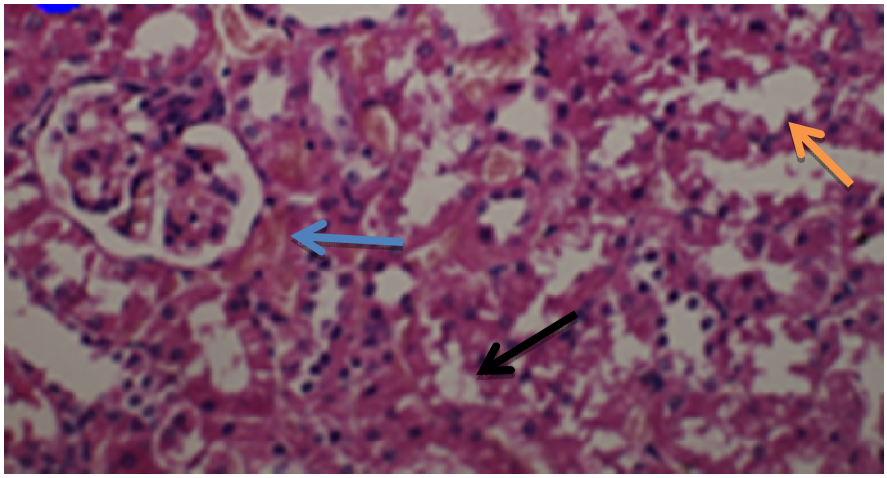

Figure (4b). kidney of a rat treated with danofloxacin showing degeneration of the epithelial lining of proximal convoluted tubules (black arrow), desquamation of tubular epithelial cells (yellow arrow), and interstitial hemorrhages (blue arrows) (H&E, X 400)

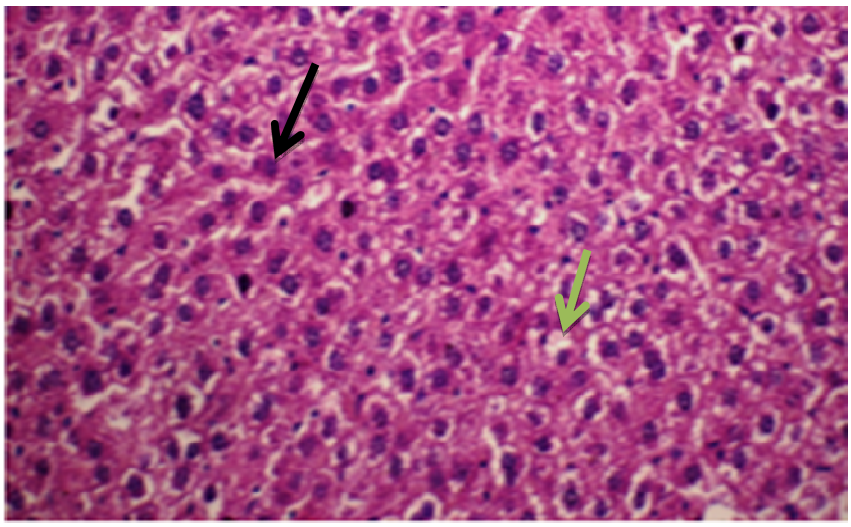

Figure (5). Liver of a rat treated with danofloxacin plus alpha- lipolic acid showing restoration of liver tissue architecture and apparently normal hepatocytes (black arrow) with very few ballooning degeneration in some hepatocytes (green arrow) (H&E, X 400)

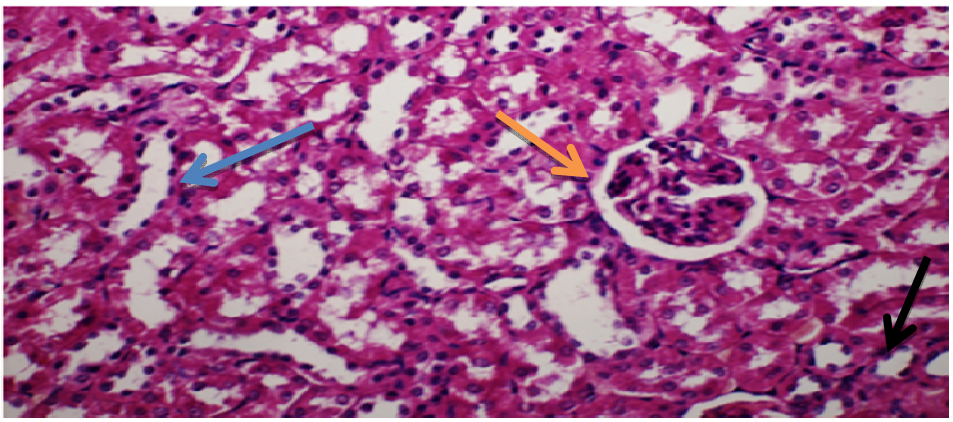

Figure (6). kidney of a rat treated with danofloxacinplus alpha- lipolic acid showing relatively restoration of normal appearance of epithelial lining of proximal convoluted tubules (black arrow), apparently normal glomerulous (yellow arrow), and relatively normal distal convoluted tubules (blue arrows) (H&E, X 400)

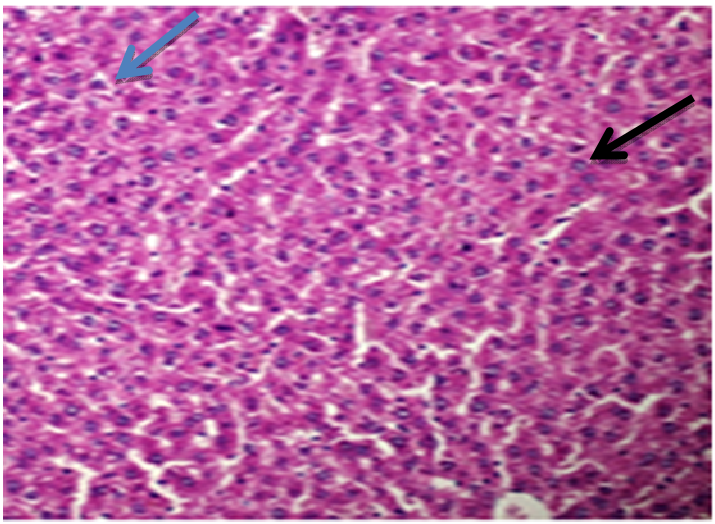

Figure (7). Liver of a rat treated with danofloxacin plus ursodeoxycholic acid showing normal hepatic architecture (black arrow) with presence of few ballooning degeneration in some hepatocytes (blue arrow) (H&E, X 200)

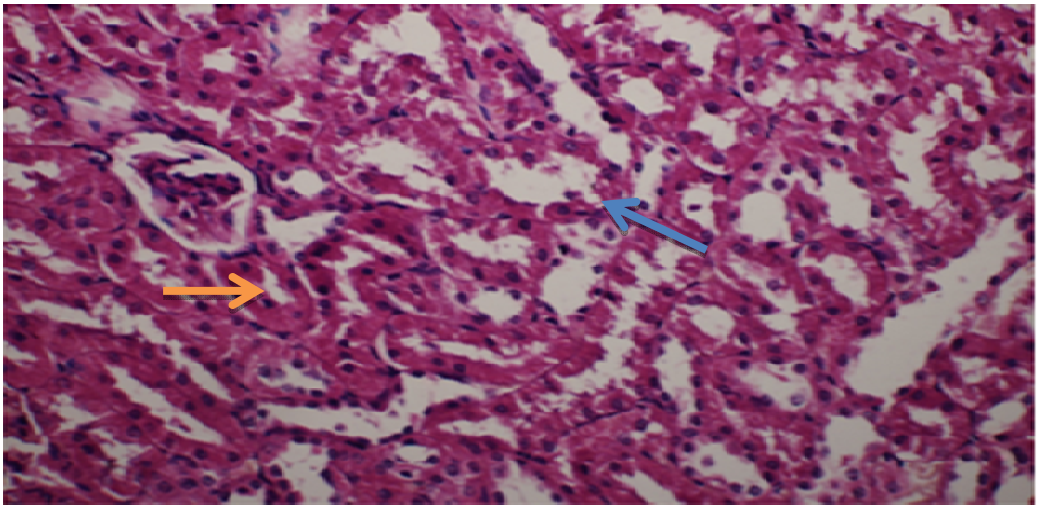

Figure (8). Kidney of a rat treated with danofloxacin plus ursodeoxycholic acid showing apparently normal renal tubules (yellow arrow) with presence of few necrotic tubular epithelial cells (the cells were swollen with deeply eosinophilic cytoplasm with karyopyknotic nuclei) (blue arrow) (H&E, X 200)

References

Abd-Elhamid, T.H.; Elgamal, D.A.; Ali, S.S.; Ali, F.E.M.; Hassanein, E.H.M.; El-Shoura, E.A.M. and Hemeida, R.A.M. (2018). Reno-protective effects of ursodeoxycholic acid against gentamicin-induced nephrotoxicity through modulation of NF-κB, eNOS and caspase-3 expressions. Cell Tissue Res., 374(2): 367-387.

Ahmed, O.A.; Randa, H.A.; Madeha, M.M. and Magda, M.Y. (2008). The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. Toxicol. 243, 261–270.

Aili, C.; Li, W.; Xia, C.; Hengjiang, G.; Shuang, C.; Xuemei, Z. and Wen, P. (2016). Ursodeoxycholic Acid Ameliorated Diabetic Nephropathy by Attenuating Hyperglycemia-Mediated Oxidative Stress. Bio. and Pharm. 39, 8.

Airey, K. and E. Koller (2003). Acute Hepatitis Associated with Levofloxacin in a Patient with Renal Insufficiency," Canad. Med. Asso. Jour., Vol. 169, p. 8.

Al-Rikably, A.; Ghadhban, R. and Majeed, S. (2016). The effects of ciprofloxacin on male rabbits Biochemical and histopathological study Al-Qadisiya. J. of Vet. Med. Sci., 15 (1): 238-344.

Aly, H.A.; Light foot, D.A. and El-Shemy, H.A. (2009). Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells in vitro. Chem. Biol. Interac., 182: 112-118.

Amita, M.; Shashank, K. and Abhay, K. (2013). Scientific Validation of the medicinal efficacy of Tinospora cordifolia. Scient. World J. Vol. 2013 Article ID 292934, 8pages.

Amudha, G.; Josephine, A. and Varalakshmi, P. (2006). Role of lipoid acid on reducing the oxidative stress induced by cyclosporine A. clinica. Chimica Acta 372, 134-139.

Bachrach, W.H. and Hofmann A.F. (1982). Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. Part I. Dig. Dis. Sci., 27, 737–761.

Bae, K.H.; Min, A.K.; Kim, J.G.; Lee, I.K. and ParkK, G. (2014). Alpha lipoic acid induces hepatic fibroblast growth factor 21 expression via upregulation of CREBH. Biochem. Biophys. Res. Commun., 455(3-4): 212-217.

Bancroft, J.D. and Layton, C. (2012). Hematoxylins and eosin In: Bancroft theory and practice of histological techniques. Suvarna, S.K. Layton, C. and Bancroft, J.D. (eds), Churchill Livingstone, New york, U.S.A,7 thed. P.P. 173-187.

Borges, L.P.; Brandão, R.; Godoi, B.; Nogueira, C.W. and Zeni, G. (2008). Oral administration of diphenyldiselenide protects against cadmium-induced liver damage in rats. Chem. Biol. Interact. 171, 15–25.

Carrascosa, F.; Lucena, M.I.; Andrade, R.J.; Caviedes, J.S.; Lavin, A.C.; Mones, J.C.; Vicente, A.P.; Serrano, B. and Serrano, V.B. (2009). Fatal Acute hepatitis after sequential treatment with levofloxacin, doxycycline, and naproxenin a patient presenting with acute mycoplasma pneumonia-infection. Clinic. Therap., Vol. 31, No. 5,1014-1019.

Chen, X.; Xu, J.; Zhang, C.; Yu, T.; Wang, H.; Zhao, M. and Xu, D. (2011). The protective effects of ursodeoxycholic acid on isoniazid plus rifampicin induced liver injury in mice. Eur. J. Clin. Pharmacol.; 659: 53–60.

Clinical and Laboratory Standards Institute (CLSI) (2008). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard-third edition. CLSI document M31-A3. Wayne, Pennsylvania, USA.

Dirk, L.R.; Alun, C.T.; Sven, T.T. and Sheri, L.M. (1992). In vitro Susceptibility of Avian Escherichia coli and Pasteurella multocida to Danofloxacin and Five Other Antimicrobials. Avi. Dis. 36: 964-967.

Dogan, Z.; Elbe, H.; Taslidere, E.; Soysal, H.; Cetin, A. and Demirtas, S. (2017). Effects of ciprofloxacin on fetal rat liver during pregnancy and protective effects of Quercetin. Biotech. and Histochem. J., 1 –6.

Ebenezer, T.O.; Ayokanmi, O. and Olaniyi, S.O. (2015). Influence of different doses of levofloxacin on antioxidant defense Systems and Markers of Renal and Hepatic Dysfunctions in Rats. Adv. in Toxicol. 1-7.

Elamaran, A.; Hariharan, P.; Ramesh, S. and Vairamuthu, S. (2015). Enrofloxacin induced oxidative stress and its amelioration with antioxidants in liver homogenates of broilers. Ind. J. Vet. and Anim. Sci. Res., 44 (2): 124-128.

Elbe, H.; Dogan, Z.; Taslidere, E.; Cetin, A. and Turkoz, Y. (2016). Beneficial effects of quercetin on renal injury and oxidative stress caused by ciprofloxacin in rats. A histological and biochemical study. Hum. and Experim. Toxicol., 35 (2).

El-Beshbishy, H.A.; Aly, H.A.A. and El-Shafey, M. (2012). Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. Toxicol. Ind. Heal., 29, 875-887.

El-Sherbiny, G.; Taye, A. and Abdel-Raheem, I. (2009). Role of ursodeoxycholic

acid in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats. Ann. Hepatol.; 8: 134–140.

EMA (The European Agency For The Evaluation Of Medicinal Products - Committee For Veterinary Medicinal Products) (1997). Danofloxacin summary report (2). EMEA/MRL / 254 / 97-final . Sept.

Evans, J.L. and Goldfine, I.D. (2000). Lipoic acid: a multifunctional antioxidant that improves insulin sensitivity in patients with type 2 diabetes. Diab. Technol. Therap., 2, 401-413.

Eucast: European committee on antimicrobial susceptibility testing (2017). breakpoint tables for interpretation of MIC_S and zone diameters. version 7.1.

Gamal, A.; Ashraf, T. and Ihab, T. (2009). Role of ursodeoxycholic acid in prevention of hepatotoxicity caused by amoxicillin-clavulanic acid in rats. Annals of Hepatol., 8 (2): 134-140.

Hannan, P.C.T.; Windsor, G.D.; Jong, A.; Schmeer, N. and Stegemann, M. (1997). Comparative susceptibilities of various animal pathogenic mycoplasmas to fluoroquinolones. Antimicrob. Ag. and Chemo-ther., 41, 2037–2040.

Hirsh, A. and Lundquist, I.B. (2009). Therapeutic effects of ciprofloxacin on the pharmacokinetics of carbamezepine in healthy adult. J. of Pharmaceut., 24(l): 63-68.

Hootkins, R.; Fenves, A.Z. and Stephens, M.K. (1989). Acute renal failure secondary to oral ciprofloxacin therapy, a presentation of three cases and a review of the literature. Clin. Nephrol.,32(2): 75-78.

Hsiao, C.J.; Younis, H. and Boelsterli, U.A. (2010). Trovafloxacin, a fluoroquinolone antibiotic with hepatotoxic potential, causes mitochondrial peroxynitrite stress in a mouse model of underlying mitochondrial dysfunction. Chemico-Biologic. Inter., 188(1): 204–213.

Ihcène, B.; Asma, B.; Amel, B.; Abdelfattah, E.F. and Mahfoud, M. (2014). Antioxidant Effect of Alpha Lipoic Acid on Hepatotoxicity Induced by Aluminium Chloride in Rats. Int. J. Pharm. Sci. Rev. Res., 29(2).19-25.

Itoh, M.; Wada, K.; Tan, S.; Kitano, Y.; Kai, J. and Makino, I. (1999). Antibacterial action of bile acids against Helicobacter pylori and changes in its ultrastructural morphology: effect of unconjugated dihydroxy bile acid. J. Gastroenterol., 34(5): 571-576.

Khaled, A.A.; Sally, A.E.A.; Wafaa, I.E.I. and Ezz-El-Din, S.E.D. (2014). Protective effects of ursodeoxycholic acid on ceftriaxone-induced hepatic injury in rats. Bull. of Fac. of Pharm., Cairo Univ., 52, 1, 45- 50.

Kim, H.Y. (2014). Analysis of variance (anova) companing means of more than two groups .restorative dentistry and endodontics , ISSN 2234-7658.

Kotb, M.A. (2008). Ursodeoxycholic acid in neonatal hepatitis and infantile paucity of intrahepatic bile ducts: Review of a historical cohort. Dig. Dis. Sci. 53(12): 3112-3118.

Lomaestro, B.M. (2000). Fluoroquinolone-induced renal failure. Drug Saf.; 22(6): 479-85.

Lukivskaya, O.; Zavodnik, L.; Knas, M. and Buko, V. (2006). Antioxidant mechanism of hepatoprotection by ursodeoxycholic acid in experimental alcoholic steatohepatitis. Adv. Med. Sci., 51: 54–9.

Mandell, L. and Tillotson, G. (2002). Safety of fluoroquinolones: An update. Can. J. Infect. Dis., 13 (1): 54–61.

Matsubar, R.; Kibe, T. and Nomura, T. (2016). Crystalline nephropathy caused by tosufloxacin. Pediat. Intern, 58(11): 1219–1221.

Mitsuyoshi, H.; Nakashima, T.; Sumida, Y.; Yoh, T.; Nakajima, Y.; Ishikawa, H.; Inaba, K.; Sakamoto, Y.; Okanoue, T. and Kashima, K. (1999). Ursodeoxycholic acid protects hepatocytes against oxidative injury via induction of antioxidants. Biochem. Biophys. Res. Commun., 263, 537–542.

Mohamed, A.H.; Ezzeldein, S.M.E.; Ashraf, K.B.; Nabila, S.H.; Soher, E.A. and Mosaad, A.A. (2014). Hepatoprotective Effect of Estradiol and alpha Lipoic Acid in Rats. Glob. J. of Pharmacol., 8 (4): 694-702.

Morakinyo, A.O.; Oludare, G.O.; Anifowose, A.A. and Adegoke, O.A. (2012). Protective Effects of Alpha Lipoic Acid on Carbon Tetrachloride-Induced Liver and Kidney Damage in Rats. British Journal of Pharmacology and Toxicology 3(1): 21-28.

Mouton, R. and Holder, K. (2006). Laboratory tests of renal function. Anaesth. and Intens. Care Med., 7, 7, 240 – 243.

Murugavel, P. and Pari, L. (2004). Attenuation of chloroquine – induced renal damage by alpha – lipoic acid: possible antioxidant mechanism. Ren. Fail. 26, 517 – 524

Nada, N.A. (2012). Possible histological changes induced by therapeutic doses of ciprofloxacin in liver and kidney of juvenile rats. Pharmacol., 3 (9): 277-480.

Nadia, H.I. (2006). Assessment of histopathological and histochemical changes in liver of pregnant female rats and their fetuses following ciprofloxacin administration. J. Egypt. Soc. Toxicol., 35,7-17.

Neer, T.M. (1988). Clinical pharmacologic features fluoroquinolone antimicrobial drugs. J. of Am. Vet. Med. Assoc. 193, 577-580.

Nikolić, R.S.; Krstić, N.S.; Nikolić, G.M. and Anđelković, D.H. (2014). Molecular mechanisms of beneficial effects of lipoic acid in copper intoxicated rats assessment by FTIR and ESI-MS. Polyhed., 80: 223-227.

Nnodim, J.; Emejulu, A.; Amaechi, A. and Nwosu Njoku, E.C. (2010). Alterations in biochemical parameters of Wistar rats administered with sulfadoxine and pyrimethamine (Fansidar). Al Ameen J. Med. Sci., 3 (4): 317 -321.

Norcia, L.J.; Silvia, A.M. and Hayashi, S.F. (1999). Studies on time-kill kinetics of different classes of antibiotics against veterinary pathogenic bacteria including Pasteurella, Actinobacillus and Escherichia coli. J. of Antibiot., 52, 52–60.

Ohkawa, H.; Ohishi, W. and Yagi, K. (1979). Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-353.

Packer, L.; Kraemer, K. and Rimbach, G. (2001). Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrit., 17: 888-895.

Perez, C.; Pauli, M. and Bazergue, P. (1990). An antibacterial assay by agar well diffusion method. Acta Bio. Et. Med. Exp. 15, 113-115.

Poupon, R.E.; Eschwège, E. and Poupon, R. (1990). Ursodeoxycholic acid for the treatment of primary biliary cirrhosis. Interim analysis of a double-blind multicenter randomized trial. The UDCA- PBC Study Group. J Hepatol., 11(1): 16-21.

Prahalathan, C.; Selvakumar, E. and Varalakshmi, P. (2006). Role of lipoic acid on adriamycin-induced testicular injury. Chem. Biol. Int. 160, 108-114.

Pratt, D.S. and Kaplan, M.M. (2000). Evaluation of abnormal liver-enzyme results in asymptomatic patients. N. Eng. J. Med., 27; 342(17): 1266–1271.

Rashwan, N.M. and Anfenan, M.L.K. (2012). Free Radical Scavenger Effects of Licorice on the Experimental Rats. J. of Appl. Scienc. Resear. 8, 4704-4710.

Ravikumar, C.; Jagadeesh, S.S.; Shivashankar, B.P.; Sunilchandra, U.; Narayanaswamy, Shridhar N.B. and Ramachandra, S.G. (2017). Histopathology of Levofloxacin Induced Toxicity in Kidney and Heart after Repeated Oral Administration in Dual Purpose Chicken. Bull. of Env., Pharmacol. and Life Sci., 6 [9], 64-69**.**

Rawi, S.M.; MouradI, M.; Arafa, N.M.S. and Alazabi, N.I. (2011). Effect of ciprofloxacin and levofloxacin on some oxidative stress parameters in brain regions of male albino rats. Af. J. of Pharm. and Pharm., 5 (16), 1888-1897.

Sandhya, P. and Varalakshmi, P. (1997). Effect of lipoic acid administration on gentamicin-induced lipid peroxidation in rats. J. Applied Toxicol., 17: 405-408.

Shila, S.; Kokilavani, V.; Subathra, M. and Panneerselvam, C. (2005). Brain regional responses in antioxidant system to α-lipoic acid inarsenic intoxicat. rat. Toxicol., 210, 2005, 25-36.

Sola, S.; Aranha, M.M.; Steer, C.J. and Rodrigues, C.M. (2007) . Mitochondrial apoptosis and the therapeutic potential of ursodeoxycholic acid. Curr. Iss. Mol . Biol. , 9 , 123–9.

Somani, S.M.; Husain, K.; Whitworth, C.; Trammell, G.L.; Malafa, M. and Rybak, L.P. (2000). Dose – dependent protection by lipoid acid against cisplatin – induced nephrotoxicity in rats. Antioxidant defense system. Pharmacol. Toxicol., 86. 234-241.

Sudheesh, N.P.; Ajith, T.A. and Janardhanan, K.K. (2013). Hepatoprotective effects of DL-α-lipoic acid and α-Tocopherol through amelioration of the mitochondrial oxidative stress in acetaminophen challenged rats. Toxicol. Mech. Meth., 23(5): 368-376.

Talla, V. and Veerareddy, P. (2011). Oxidative stress induced by fluoroquinolones on treatment for complicated urinary tract infections in Indian patients. J. Yo. Pharm.; 3(4): 304-309.

Tamura, M.; Oschino, N. and Chance, B. (1982). Some characteristics of hydrogen and alkylhydro peroxides metabolizing system in cardiac tissues. J. Biochem., 92: 1019-1031 .

Taslidere, E.; Dogma, Z.; Elbe, H.; Verdi, N.; Cetin, A. and Turkoz, Y. (2016). Quercetin protection against ciprofloxacin induced liver damage in rats. Biotechn. and Histochem., 91(2): 116-121.

Trasë, B.; Maden, M.; Basë, A.L.; Elmas, M.; Yazar, E. and Civelek, T. (2001). Investigation of Biochemical and Haematological Side effects of Enrofloxacin in Dogs. J. Vet. Med. A 48, 59-63.

Vanni, M.; Meucci, V.; Tognetti, R.; Cagnardi, P.; Montesissa, C.; Piccirillo, A.; Rossi, A.M.; Bello, D.D. and Intorre, L. (2014). Fluoroquinolone resistance and molecular characterization of gyrA and parC quinolone resistance-determining regionsin Escherichia coli isolated from poultry. Poul. Sci., 93: 856–863.

Wolfson, J.S. and Hooper, D.C. (1991). Overview of fluoroquinolone safety. Am. J. Med., 91 (Suppl 6A): 153S-61S.

World Health Organization (WHO), (1998). Use of Quinolones in Food Animals and Potential Impact on human health. WHO/EMC/ ZDI/98.10

Xia, X.; Lin, P.; Li-Jie, J.; Wei-Xuan, L.; Jia -Yu, X.; Yon-Jia, J. and Zhi-Qiang, W. (2018). In Vivo Pharmacokinetic/ Pharmacodynamic Profiles of Danofloxacin in Rabbits Infected With *Salmonella typhimurium* After Oral Administration. Front. in pharmacol., 9, 1-8.

Xie, H.J.; Broberg, U.G.L.; Lundgren, S.C.S.; Meurling, P.C. and Rane, A.H.M. (2003). Alteration of pharmacokinetics of cyclophosphamide and suppression of the cytochrome P450 genes by ciprofloxacin. Bone Marrow Transplant. 31: 197-203.

Yasser, F.A.; Omar, S.D.; Nabila, S.S. and Khairy, M.E. (2015). Assessment of the role of a-lipoic acid against the oxidative stress of induced iron overload. J.of Rad. Res. and Appl. Sci. 8, Issue 1, P: 26-35.

Yoshikawa, M.; Tsujii, T.; Matsumura, K.; Yamao, J.; Matsumura, Y.; Kubo, R.; Fukui, H. and Ishizaka, S. (1992). Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatol.,16: 358–64.

Zulfiqar, Q.B.; Muhammad, A.R.; Shabber, A.A. and Sumera, B. (2017). Ciprofloxacin-induced Hepatotoxicity in a Healthy Young Adult. Cureus, 9(2): e1016.