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Some Pharmacogical Adverse Effects of Moxifloxacin and Staphylococcal Aureus in Rats

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Abstract

Moxifloxacin is a broad spectrum fluoroquinolone antibacterial agent. We examined the plasma biomarkers of hepatorenal adverse effects of moxifloxacin ,lipid profile and blood glucose in rat following administration of moxifloxacin (MXF). Thirty-six Wistar rats, 200–250 g, were randomized into four groups (I–IV). Animals in group I (control) received 3.3 mL of distilled water, while animals in groups II administrated with moxifloxacin received 3.3 mL each of MXF equivalent to 26.4 mg/kg b.wt respectively for five successive days, III while animals in this group experimentally infected with staphylococcal aureus, and IV while animals in this group first experimentally infected with staphylococcal infection then received 3.3 mL each of moxifloxacine equivalent to 26.4 mg/kg b.wt respectively for five successive days. After seven days, plasma urea, bilirubin, and creatinine were significantly elevated in the MXF-treated animals. Activities of alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were significantly increased in the plasma of MXF-treated animals compared to control. Also plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides increased significantly in the MXF-treated groups relative to control. Moreover, a significant increase in serum glucose concentration ,a significant decrease in blood Hb concentration and white blood cells)WBCs concentration (key words,MXF moxifloxacin ,HDL high density lipoprotein ,LDL low density lipoprotein .

Keywords: MXF moxifloxacin , HDL high density lipoprotein, LDL low density lipoprotein.

1. Introduction

Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent with a broad spectrum of bactericidal action. possess enhanced activity against Gram-positive bacteria, most notably against penicillin-susceptible and penicillin-resistant strains of S. pneumoniae. It is available for oral and intravenous administration, respectively, as a once-daily 400 mg antibiotic for the treatment of respiratory tract infections, chronic bronchitis, and acute bacterial sinusitis and in some cases pelvic inflammatory disease, complicated and uncomplicated skin and skin structure infections, complicated intra-abdominal infections, ocular bacterial keratitis, and community acquired pneumonia [1–3].

Moxifloxacin like other quinolones have a bicyclic aromatic core with a carbon at position 8 and demonstrate an N-1 cyclopropyl moiety

Moxifloxacin hydrochloride (1-cyclopropyl-7-[(S,S)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinoline carboxylic acid ·HCl

Following oral administration, Moxifloxacin is well absorbed from the gastrointestinal tract with approximately 50% bound to serum proteins . It binds weakly to plasma proteins and penetrates well into most tissue and fluid compartments. MXF is metabolized to an N-sulfate conjugate and an acyl glucuronide in humans[4]. Like other fluoroquinolones, MXF exhibit bactericidal activity by binding to bacterial topoisomerases II (DNA gyrase) and topoisomerase IV [5] .By binding to these enzymes, the fluoroquinolones interfere with DNA replication, repair, and transcription, resulting in bacterial death. The ability to target both enzymes has been promoted as a major advantage of the fluoroquinolones in preventing or delaying the emergence of resistance[6] .

2. Materials and methods

2.1 Materials

2.1.1 Chemicals and drugs

A- Moxifloxacin

was obtained from EL-NILE company for pharmaceuticals and chemical industries for EVAPHARMA, Egypt in a form of hydrochloric acid . Moxifloxacin was suspended into sodium chloride at a concentraion of 400 mg / 250 ml .

B- Staphylococcus Areus

Staphylococcus aureus were obtained from Research Institute of Animal Health,Cairo,Egypt.The indicator strains were activated on activated on tryptic soya broth (TSB) which obtained from International Diagnostic Group LABM, U.K. were incubated at 37° C for 24 hr.The activated strains were cultivated and maintained in nutrient agar slants .The agar slants were preserved in a refrigerator at 4° C until use .The organisms were activated for three successive subculture till obtaining the concentration of 10^7 cfu / ml

2.1.2 Instrument

1- Neubour's haemocytometer (for RBCs and WBCs count).
2- Centrifuge (MLWT 52-1 Germany) was used for separating serum.
3- Wintrobe haematocrit tubes for packed cell volume determination.

4- Deep freeze (-20 co) (Sital co., A.R.E.) was used for keeping serum samples.
5- Microtome (American Optical Company U.S.A) was used for sectioning the tissues for histopathologyical studies.
6- Ordinary microscope (Car I-Zeiss 0.65) was used for histopathological examinations.
7- Other equipments: beakers, bottles, cotton, flasks, forcipes, gauze, graduated cylinders, scissors and syringes were used.

2.1.3 Experimental animals

Thirty six male Wister albino rats weighting 200-250 g were used in the experimental investigation of these study. Rats were obtained from animal house of Faculty of Veterinary Medicine, Benha university .The animals were housed in 49x35 cm stainless steel wire mesh cages with bedding of ground wood chips at 21 c. They were fed fresh-pelleted food and their water as placed in glass bottles of 500 ml. Rats were kept at a constant environmental and nutritional condition throughout the period of experiment. The animals were left for 15 days for acclimatization before the beginning of the experiment.

2.1.4 Ration and additives

The animal were fed on a constant ration in the form of concentrated diet composed of carbohydrate 58%, protein 17.5%, lipid 3.4%, cellulose 3.1%, minerals 1.49%, phosphorus 0.59% and moisture 12%.

2.2 Methods

2.2.1 Experimental design

The rats were randomly divided into main 4 groups.

Group (1): Served as a negative control group.Nine rats were administrated 3.3 ml of normal saline per rat weighting 200 g orally for 5 succeccive days.

Group (2):.Nine rats were administrated moxifloxacin which dissolved into sodium chloride at a concentration of 400 mg/ 250 ml administrated orally once daily for 5 succeccive days at dose of 26.4mg / k.g. of b.wt of rat . **(7)**

Group(3): Nine rats were infected with fresh mixture of staphylococcus aureus which administrated Intraperitoneally by dose 1.5 ml / k.g. of b.wt of rat at conc of 10^7 cfu (colony forming units) per ml for induction of bacterial infection

Group(4): Nine rats were infected with staphylococcus aureus
1.5 ml / **k**.g. of b.wt of rat at conc of 107 cfu (colony forming units) per ml for induction of bacterial infection followed by administration of moxifloxacin orally 26.4mg / k.g. of b.wt of rat once daily for 5 succeccive days .

2.2.2 Blood samples

Blood samples were taken at first, seventh and fourteenth day post-treatment in all groups . Two blood samples were taken from each rat in the group for both biochemical and hematological studies from median canthus of the eye.

The first blood sample

was collected without anticoagulant for separation of clear serum for biochemical analysis. These serum samples were used for biochemical analysis to determine serum total bilirubin, serum transaminases activities (AST and ALT), alkaline phosphatase (ALP), total protein, albumin, blood creatinine, blood urea, creatinine kinase,

The second sample of blood

was collected in the test tube mixed with sodium citrate 3.8% as anticoagulant . The sample was shaked several times to ensure mixing of blood with anticoagulant. These blood samples were used for hematological studies to determine erythrocytic count, total leucocytic count, haemoglobin concentration.

2.2.3 Histopathological samples

The treated rats were sacrificed at first day, seventh day and fourteenth day. Specimens were collected from liver, kidney and spleen from each sacrificed tested rats and fixed directly in formalin solution 10% .

2.2.4 Serum biochemical analysis

2.2.4.1 Liver function tests

1) Determination of total bilirubin

Serum total and direct bilirubin was determined according to the method described by Jendrasski (1938).

2) Determination of serum aspartate aminotransferase activity (AST)

Serum aspartate aminotransferase was determined colorimeterically using spectrophotometer using specific kits (Centronic Company) according to (Reitman and Frankel (1957).

3) Determination of serum alanine aminotranferase activity (ALT)

Determination of serum alanine aminotransferase was carried out by using a spectrophotometer of using specific kits (Centronic Company) according to Reitman and Frankel (1957).

4) Determination of serum alkaline phosphatase (ALP)

Determination of serum alkaline phosphatase was according to Chariman (1983).

5) Determination of serum total protein

Colorimetric determination of total protein level in the serum of rats was carried out using spectrophotometer, using specific kits (Centronic Company) according to Doumas (1975).

2.2.4.2 Haematological studies Blood cell count

Total erythrocytes and leucocytes were counted using the improved neubauer chambe, natt and herrick; solution as diluting fluid according to the method described by **Natt and Herrick, (1952).**

1) Erythrocytic count (RBCs) (10^6/nmm^3)

2) Total leucocytic count (WBCs) (10^3/mm^3)
3) Haemoglobin determination (HB%)

2.2.5 Histopathology studies

Tissue samples were taken from the liver and kidney of rats in different groups and fixed in formalin solution 10% for twenty four hours. Washing was done in tap water then serial dilution of alcohol (methyl, ethyl and absolute ethyl were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hour. Paraffin bees wax tissue block were prepared for sectioning at 4 microns thickness by slidge microtome .The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for routine examination through the light microscope (Banchroft., et al 1996).

2.3Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS 16 Inc. Released, 2009) to determine if variables differed among groups, according to Snedecor and Cochran (1998). The Sharipo-Willk test was used to test the normal distribution of the data before statistical analysis was performed . Comparison among means was conducted by one-way ANOVA and subsequent Ducann's multiple range test (Ducan, 1955). Probability values of less than 5% ($p \leq 0.05$) were considered significant.

3. Results

The pharmacological effect of Moxifloxacin administration on normal and staph. aureus infected groups of male rats, in comparison with control normal and non-treated staph. aureus infected groups, were assessed by measuring the next biochemical parameters:

A) Serum parameters

Liver markers

1-ALT, AST, and ALP activity.
2-Total bilirubin, total protein, and albumin concentration.

B) Blood parameters

Hb, RBCs, and WBCs concentrations.

` **These results were statistically analyzed and represented in (9) tables and (9)figures, as following**

1) Serum alanintransaminstrase (ALT) activity

The activity of serum alanintransaminstrase (ALT) in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in Table (1) and graphically illustrated in Fig (1).

The obtained data in Table(1) and Fig (1) showed that

1- Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum alanintransaminstrase(ALT) activity.
2- Infection of normal rats by staph. aureus exhibited a slight increase in serum alanintransaminstrase (ALT) activity at the 1st day and 7th day , while exhibited a non-significant increase at 14th days, when compared with control normal group.
3- Administration of moxifloxacin to staph. infected group of male rats exhibited a significant increase in serum alanintransaminstrase (ALT) activity after the 1st, 7th, and 14th days, when compared with staph. infected none treated group.

Table (1) Effect of moxifloxacin administration on serum alanintransaminstrase (ALT) activity (U/L) in normal and staph. infected groups of male albino rats.

Durations	ALT Activity (U/L)		
Animal Groups	At 1st day	At 7th day	At 14th day
Control normal group.	15.31 ± 0.44c	15.96 ± 1.78b	15.13 ± 0.42c
Moxifloxacin group.	25.47 ± 1.55a	32.63 ± 0.73a	22.24 ± 1.35b
Staph. infected group.	19.21 ± 0.86b	19.67 ± 1.68b	17.95 ± 1.20c
Staph. Infected + Moxifloxacin group.	28.21 ± 0.91a	36.60 ± 1.68a	26.26 ± 1.38a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

2) Serum aspartatransaminase (AST) activity

The activity of serum aspartatransaminase (AST) in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in Table (2) and graphically illustrated in Fig (2).

The obtained data in table (2) and Fig (2) showed that

1- Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum aspartatransaminase(AST) activity after the 1st, 7th and 14th days, when compared with control normal group.
2- Infection of normal rats by staph. aureus exhibited a non-significant increase in serum aspartatransaminase (AST) activity at the 1st and 14th days, while exhibited a significant increase at the 7th days, when compared with control normal group.
3- Administration of moxifloxacin to staph. infected group of male rats exhibited a significant increase in serum aspartatransaminase (AST) activity after the

1st, 7th, and 14th days, when compared with staph. infected none treated group.

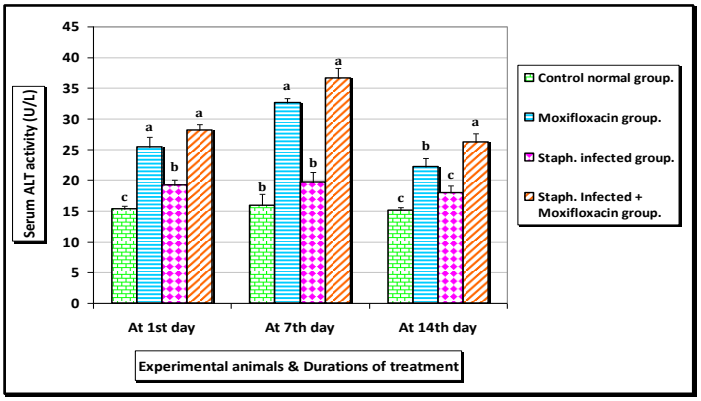

Fig (1) Activity of serum alanintransaminstrase (ALT) (U/L) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

Table (2) Effect of moxifloxacin administration on serum aspartatransaminase (AST) activity (U/L) in normal and staph. infected groups of male albino rats.

Durations	**AST Activity (U/L)**		
Animal Groups	**At 1st day**	**At 7th day**	**At 14th day**
Control normal group.	15.69 ± 1.15^{b}	16.42 ± 1.45^{d}	16.39 ± 1.68^{b}
Moxifloxacin group.	23.89 ± 2.18^{a}	29.75 ± 1.54^{b}	20.57 ± 0.75^{a}
Staph. infected group.	18.00 ± 1.05^{b}	20.47 ± 0.45^{c}	16.87 ± 0.81^{b}
Staph. Infected + Moxifloxacin group.	25.07 ± 1.08^{a}	35.09 ± 1.14^{a}	23.18 ± 0.83^{a}

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

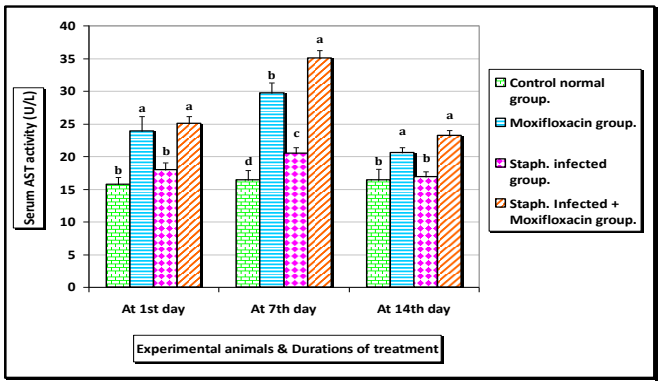

Fig (2) Activity of serum aspartatransaminase (AST) (U/L) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

3) Serum Alkaline phosphatase (ALP) activity

The activity of serum Alkaline phosphatase (ALP) in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in Table(3) and graphically illustrated in Fig (3).

The obtained data in table (3) and Fig (3) showed that

1- Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum Alkaline phosphatase(ALP) activity after the 1st, 7th and 14th days, when compared with control normal group.

2- Infection of normal rats by staph. aureus exhibited a non significant increase in serum Alkaline phosphatase (ALP) activity at the 1st, 7th and 14th days, when compared with control normal group.

3- Administration of moxifloxacin to staph. infected group of male rats exhibited a significant increase in serum Alkaline phosphatase(ALP) activity after the

1st, 7th, and 14th days, when compared with staph. infected none treated group.

Table (3) Effect of moxifloxacin administration on serum Alkaline phosphatase (ALP) activity (U/L) in normal and staph. infected groups of male albino rats.

Durations / Animal Groups	Alkaline phosphatase ALP Activity (U/L)		
	At 1st day	At 7th day	At 14th day
Control normal group.	122.15 ± 4.37b	120.65 ± 2.53b	123.81 ± 4.45b
Moxifloxacin group.	183.94 ± 4.39a	208.82 ± 2.90a	197.15 ± 2.63a
Staph. infected group.	125.79 ± 4.00b	125.04 ± 1.70b	123.04 ± 3.50b
Staph. Infected + Moxifloxacin group.	185.40 ± 5.44a	214.58 ± 4.56a	208.55 ± 2.09a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

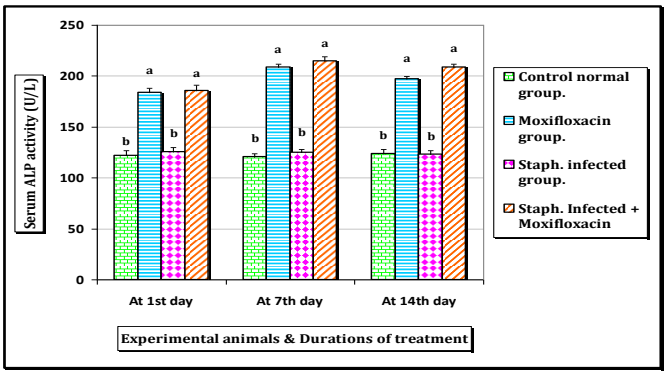

Fig (3) Activity of serum Alkaline phosphatase (ALP) (U/L) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

4) Serum Total Bilirubin Concentration

The concentration of serum total bilirubin in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in table(4) and graphically illustrated in Fig (4).

The obtained data in Table(4) and Fig (4) showed that

1- Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum total bilirubin concentration after the 1st, 7th and 14th days, when compared with control normal group.

2- Infection of normal rats by staph. aureus exhibited a non-significant increase in serum total bilirubin concentration at the 1st, 7th and 14th days, when compared with control normal group.

3- Administration of moxifloxacin to staph. infected group of male rats exhibited a significant increase in serum total bilirubin concentration after the 1st, 7th, and 14th days, when compared with staph. infected none treated group.

Table (4) Effect of moxifloxacin administration on serum total bilirubin conc. (mg/dl) in normal and staph. infected groups of male albino rats.

Durations / Animal Groups	Total Bilirubin Concentration (mg/dl)		
	At 1st day	At 7th day	At 14th day
Control normal group.	0.75 ± 0.06b	0.83 ± 0.03c	0.72 ± 0.05b
Moxifloxacin group.	1.33 ± 0.07a	1.84 ± 0.07b	1.05 ± 0.13a
Staph. infected group.	0.81 ± 0.05b	0.85 ± 0.08c	0.80 ± 0.06b
Staph. Infected + Moxifloxacin group.	1.53 ± 0.08a	2.13 ± 0.09a	1.10 ± 0.06a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

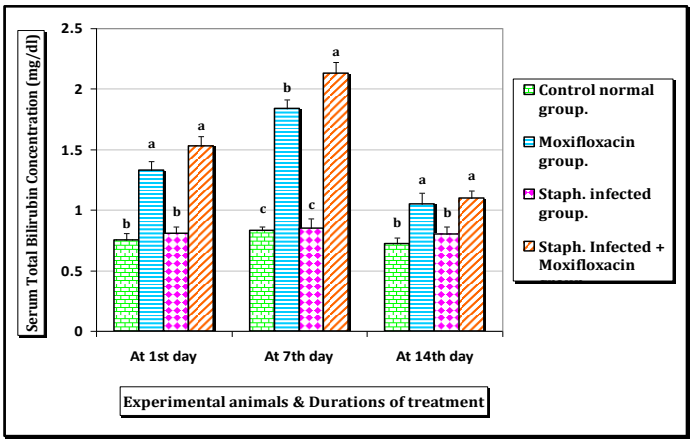

Fig (4) Concentration of serum total bilirubin (mg/dl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

5) Serum Total Protein Concentration

The concentration of serum total protein in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in table[5]and graphically illustrated in Fig (5).

The obtained data in Table (5) and Fig (5) showed that

1- Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum total protein concentration after the 1st, 7th and 14th days, when compared with control normal group.

2- Infection of normal rats by staph. aureus exhibited a non-significant decrease in serum total protein concentration at the 1st, 7th and 14th days, when compared with control normal group.

3- Administration of moxifloxacin to staph. infected group of male rats exhibited a significant increase in serum total protein concentration after the 1st, 7th, and 14th days, when compared with staph. infected none treated group.

Table (5) Effect of moxifloxacin administration on serum total protein conc. (g/dl) in normal and staph. infected groups of male albino rats.

Durations	**Total Protein Concentration (g/dl)**		
Animal Groups	At 1st day	At 7th day	At 14th day
Control normal group.	6.83 ± 0.26b	6.93 ± 0.40c	7.13 ± 0.20b
Moxifloxacin group.	11.81 ± 0.91a	15.51 ± 0.64b	9.51 ± 0.64a
Staph. infected group.	6.70 ± 0.42b	6.73 ± 0.57c	6.96 ± 0.14b
Staph. Infected + Moxifloxacin group.	14.17 ± 0.79a	18.16 ± 0.80a	10.64 ± 0.98a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

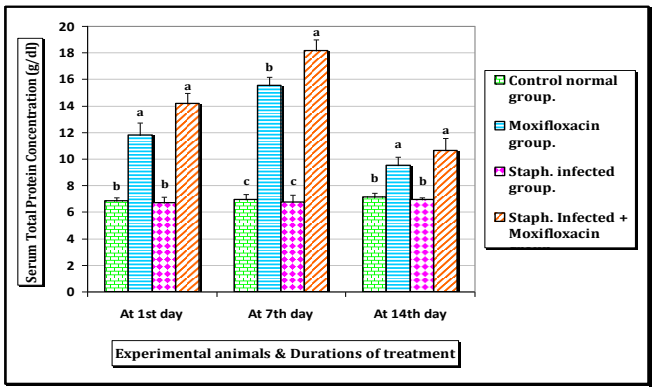

Fig (5) Concentration of serum total protein (g/dl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

6) Serum Albumin Concentration

The concentration of serum albumin in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in **Table [6]**and graphically illustrated in **Fig (6).**

The obtained data in table(6) and Fig (6) showed that

1. Administration of moxifloxacin to normal group of male rats exhibited a significant increase in serum albumin concentration after the 1st, 7th and 14th days, when compared with control normal group.
2. Infection of normal rats by staph. aureus exhibited a non-significant decrease in serum albumin concentration at the 1st, 7th and 14th days, when compared with control normal group.
3. Administration of moxifloxacin to staph. infected group of male rats exhibited a non significant increase in serum albumin concentration after the 1st, 7th, and 14th days, when compared with staph. infected non treated group.

Table (6) Effect of moxifloxacin administration on serum albumin conc. (g/dl) in normal and staph. infected groups of male albino rats.

Durations / Animal Groups	Albumin Concentration (g/dl)		
	At 1st day	At 7th day	At 14th day
Control normal group.	4.51 ± 0.13b	4.44 ± 0.16b	4.39 ± 0.14a
Moxifloxacin group.	4.78 ± 0.08a	5.06 ± 0.13a	4.58 ± 0.10a
Staph. infected group.	4.47 ± 0.11b	4.42 ± 0.06b	4.29 ± 0.07a
Staph. Infected + Moxifloxacin group.	4.90 ± 0.09a	5.05 ± 0.10a	4.53 ± 0.12a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

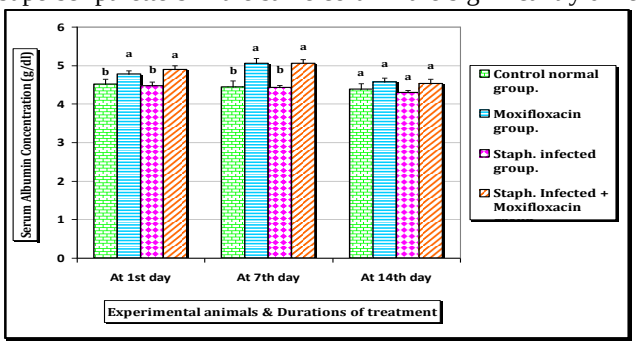

Fig (6) Concentration of serum albumin (g/dl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

7) Blood hemoglobin level (Hb) Concenration

The concentration of blood hemoglobin level(Hb) in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in Table (7) and graphically illustrated in Fig (7).

The obtained data in table (7) and Fig (7) showed that

1. Administration of moxifloxacin to normal group of male rats exhibited a significant decrease in blood Hb concentration after the 1st, 7th and 14th days, when compared with control normal group.
2. Infection of normal rats by staph. aureus exhibited a non-significant decrease in blood Hb concentration at the 1st, 7th days, while exhibited a significant decrease after 14th days, when compared with control normal group.
3. Administration of moxifloxacin to staph. infected group of male rats exhibited a significant decrease in blood Hb concentration after the 1st, 7th, and 14th days, when compared with staph. infected none treated group.

Table (7) Effect of moxifloxacin administration on blood hemoglobin level(Hb) concentration (g/dl) in normal and staph. infected groups of male albino rats.

Durations / Animal Groups	Hb concentration (g/dl)		
	At 1st day	At 7th day	At 14th day
Control normal group.	15.42 ± 0.56a	15.80 ± 1.31a	16.29 ± 1.12a
Moxifloxacin group.	10.20 ± 0.18b	9.29 ± 0.07b	10.73 ± 0.30c
Staph. infected group.	14.56 ± 0.70a	13.77 ± 0.45a	13.33 ± 0.23b
Staph. Infected + Moxifloxacin group.	10.01 ± 0.18b	9.04 ± 0.16b	11.49 ± 0.41c

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

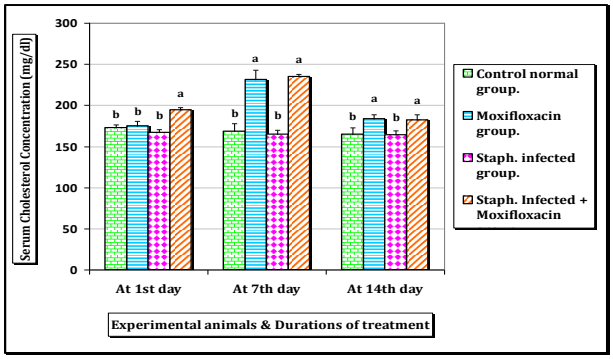

Fig (7) Concentration of blood Hb (g/dl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

8) Blood red blood cells (RBCs) Concenration

The concentration of red blood cells (RBCs)in control normal, staph. infected, moxifloxacin treated groups of male albino rats is presented in table[8] and graphically illustrated in Fig (8).

The obtained data in Table(8) and Fig (8) showed that

1. Administration of moxifloxacin to normal group of male rats exhibited a non-significant decrease in red blood cells(RBCs) concentration after the 1st, 7th and 14th days, when compared with control normal group.
2. Infection of normal rats by staph. aureus exhibited a non-significant decrease in red blood cells (RBCs) concentration at the 1st, day, while exhibited a non-significant increase after 7th and 14th days, when compared with control normal group.
3. Administration of moxifloxacin to staph. infected group of male rats exhibited a non-significant decrease in red blood cells (RBCs) concentration after the 1st, and 14th days, while exhibited a significant decrease after 7th day, when compared with staph. infected none treated group.

Table (8) Effect of moxifloxacin administration on red blood cells (RBCs)concentration (Cells/µl) in normal and staph. Infected groups of male albino rats.

Durations	red blood cells (RBCs) X 10^6 Concentration (Cells/µl)		
Animal Groups	**At 1st day**	**At 7th day**	**At 14th day**
Control normal group.	7.10 ± 0.12^a	6.82 ± 0.53^a	7.15 ± 0.64^a
Moxifloxacin group.	6.75 ± 0.20^a	4.36 ± 0.08^b	7.03 ± 0.14^a
Staph. infected group.	6.92 ± 0.52^a	7.30 ± 0.09^a	7.24 ± 0.32^a
Staph. Infected + Moxifloxacin group.	6.61 ± 0.18^a	4.09 ± 0.12^b	7.05 ± 0.23^a

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

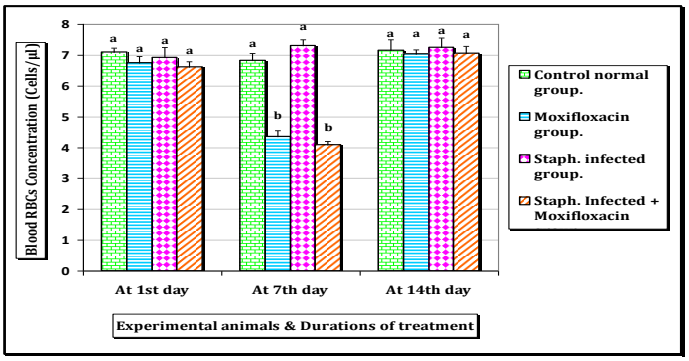

Fig (8) Concentration of blood red blood cells (RBCs) (Cells/µl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats

9. Blood white blood cells (WBCs) Concenration

The concentration of white blood cells (WBCs) in control normal, staph. infected, moxifloxacin treated

groups of male albino rats is presented in Table (9) and graphically illustrated in Fig (9) .

The obtained data in table (9) and Fig (9) showed that

1. Administration of moxifloxacin to normal group of male rats exhibited a significant decrease in white blood cells (WBCs) concentration after the 1st, 7th and 14th days, when compared with control normal group.
2. Infection of normal rats by staph. aureus exhibited a significant increase in white blood cells (WBCs) concentration after the 1st, 7th and 14th days, when compared with control normal group.
3. Administration of moxifloxacin to staph. infected group of male rats exhibited a significant decrease in white blood cells (WBCs)concentration after the 1st, 7th, 14th days, when compared with staph. infected none treated group.

Table (9) Effect of moxifloxacin administration on white blood cells (WBCs) concentration (Cells/µl) in normal and staph. infected groups of male albino rats.

Durations / Animal Groups	(white blood cells) WBCs X 103 Concentration (Cells/µl) At 1st day	At 7th day	At 14th day
Control normal group.	8.78 ± 0.16^{c}	8.64 ± 0.39^{c}	8.92 ± 0.34^{b}
Moxifloxacin group.	6.54 ± 0.37^{d}	5.65 ± 0.26^{d}	7.48 ± 0.37^{c}
Staph. infected group.	14.72 ± 0.61^{a}	18.44 ± 0.79^{a}	14.00 ± 0.25^{a}
Staph. Infected + Moxifloxacin group.	12.71 ± 0.35^{b}	10.88 ± 0.24^{b}	9.25 ± 0.31^{b}

- Data are presented as (Mean±S.E), S.E= standard error.
- Mean values with different superscript letters in the same column are significantly different at (P>0.05).

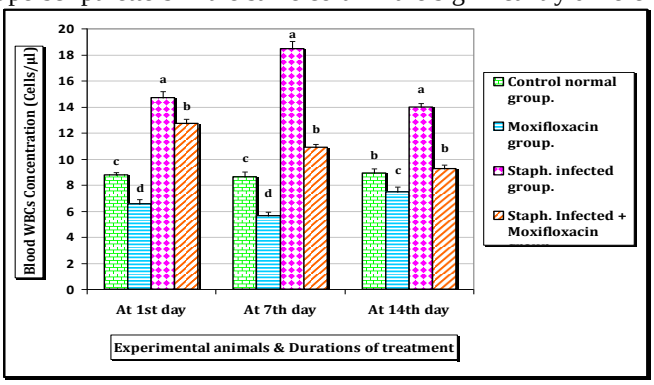

Fig (9) Concentration of white blood cells (WBCs)(Cells/µl) in control normal, staph. infected, and moxifloxacin treated groups of male albino rats.

C. Histopathological findings

Histopathological findings of liver

The Liver of moxifloxacin treated rats (group 2) showed hydropic degeneration of hepatocytes after one day of treatment .Meanwhile after seven days from the treatment with moxifloxacin, congestion of portal blood vessels with clear vacuolation of hepatocyte were detected .Moreover, after fourteen days of the treatment, the liver showed marked dilatation of central vein with severe degree of hydropic degeneration of hepatocyte.

The effect of therapeutic dose of moxifloxacin (26.4mg/ kg. body weight) orally for 5 successive days on normal and staphylococcus aureus infected groups of male rats in comparison with control normal and non-treated staphylococcus aureus infected groups were assessed by the next histopathological findings.

Microscopical examination of different sections taken from specimens of liver and kidney of control rats revealed normal histopathological structure.

Liver of control rats (group1) showed normal appearance of hepatocytes arranged into cords radiating from the central vein and were separated by blood sinusoids **Fig (10)**.

The Liver of moxifloxacin treated rats (group 2) showed hydropic degeneration of hepatocytes after one day of treatment.Meanwhile after seven days from the treatment with moxifloxacin, congestion of portal blood vessels with clear vacuolation of hepatocyte were detected Fig (11). Moreover, after fourteen days of the treatment, the liver showed marked dilatation of central vein with severe degree of hydropic degeneration of hepatocyte Fig (12) .

While the liver of rat the with Staphylococcus aureus and nontreated with moxifloxacin (group3) showed after one day from the infection mild congestion and dilatation of the central vein and hepatic sinusoid and vacuolation in cytoplasm of hepatocytes. Moreover, the hepatocytes were also suffered from mild degenerative changes in form of small vacuoles in their cytoplasm. Intravascular leukocytic infiltration were also detected Fig(13,14)While after seven days from the infection, the liver of this rat suffered from severe degree of

degenerative changes in the form of severe degree of hydropic degeneration in the hepatocyte with severe dilatation of the central vein and activation of Von-Kupffer cells Fig(15). Moreover, mild hyperplastic proliferation of epithelium lining the bile duct with few periductal leukocytic infiltration were also detected Fig(16).Meanwhile after fourteen days from the infection, degenerative changes were observed in the liver.

While the liver of the infected rats with Staphylococcus aureus followed by moxifloxacin treatment (group 4) showed other lesions. The liver of rats in this group after one day from the infection and treatment showed no pathological alterations. But after seven days from the infection and treatment, the liver showed congestion of central vein and hepatic sinusoids. The hepatocytes were suffered from mild degenerative changes manifested by minute vacuoles in the hepatocyte Fig(17). In some examined sections of the liver of these rats after fourteen day from treatment of infected rats followed by treatment with moxifloxacin showed severe thickening and hyalinization of the portal blood vessels with mild intravascular leukocytic infiltration Fig (18,19). Focal mononuclear and polymorphonuclear leukocytes were also noted in the hepatic parenchyma Fig[20].After fourteen days from the treatment of infected rats, the bile ducts showed hyperplasia of epithelial cells lining with normal histological structure of the hepatocyte Fig(21,22,23).

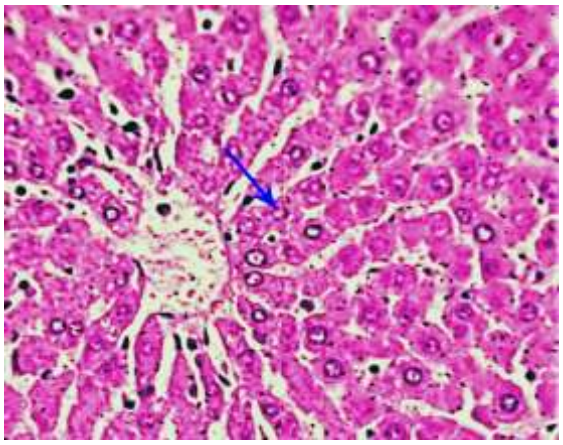

Fig (10) Liver of rat in control group (group 1) showing normal histological structure of the central vein (cv) and hepatic cords (arrow). (H&E, X200).

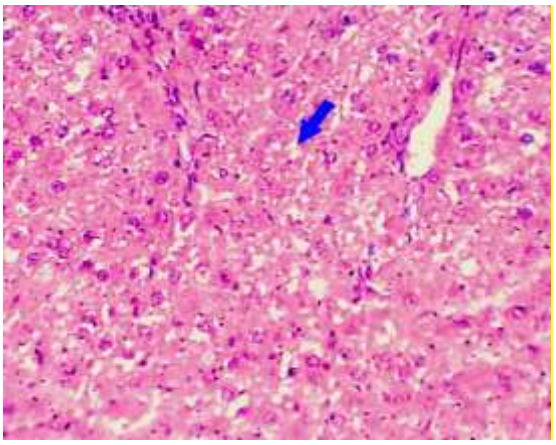

Fig (11) Liver of rat treated with moxifloxacin 26.4 for five successive days (group2). Liver after one day from treatment showing hydropic degeneration of hepatocyte (H&E,X 200).

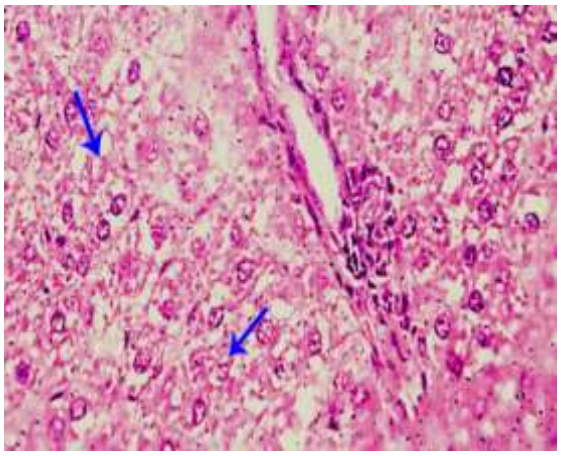

Fig (12) High power of the previous figure showed hydropic degeneration of hepatocyte **(**H&E, X400).

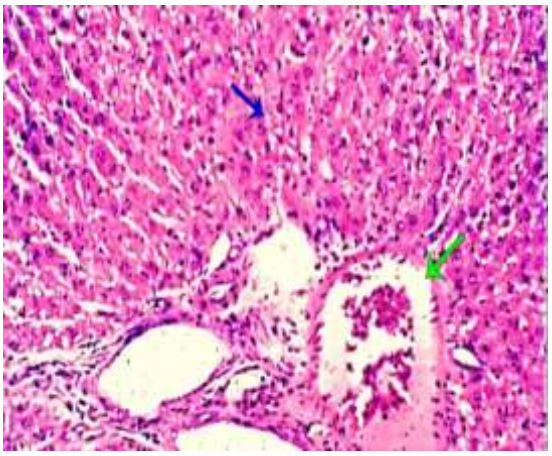

Fig (13) Liver of rat treated with moxifloxacin 26.4 for five successive days (group2) showing after seven day of treatment congestion of portal blood vessels with clear vacuolation of hepatocyte. **(**H&E, X 200).

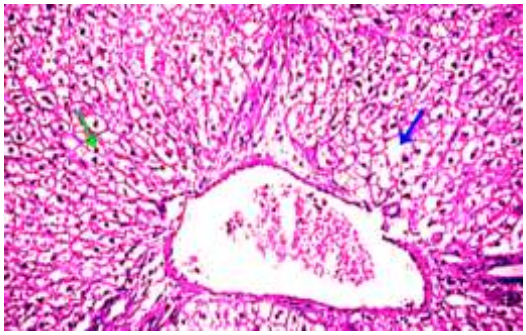

Fig (14) Liver of rat treated with moxifloxacin 26.4 for five successive days (group2); Liver after fourteen days from treatment showed severe degree of hydropic degeneration of hepatocytes with pyknosis of their nuclei, dilatation and congestion of the central vein (C) (H,Ex400).

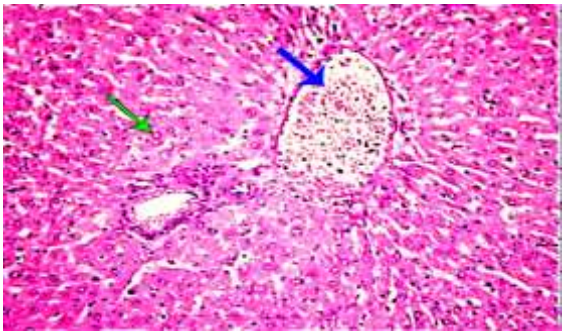

Fig (15) Liver of rat infected with staphylococcus aureus at conc of 10^7 cfu (group 3). After one day from infection showing congestion and dilatation of the central vein and vacuolation in the cytoplasm of hepatocytes. (H&E, x200)

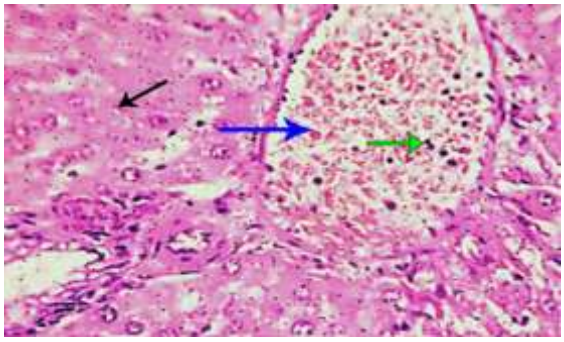

Fig (16) High power of the previous figure showing severe congestion and intravascular leukocytic infiltration in the portal blood vessels and vacuolation in the cytoplasm of hepatocytes . (H&E, x400)

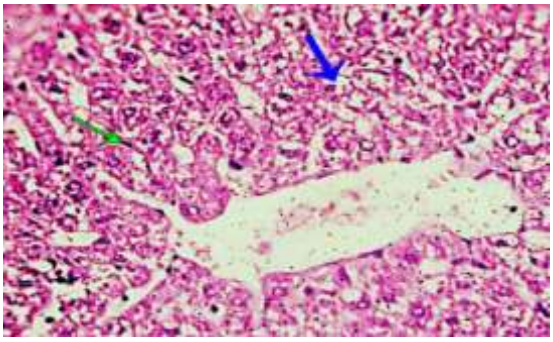

Fig (17) Liver of rat infected with staphylococcus aureus at conc of 10^7 cfu) (group3). After seven days liver showing severe degree of degenerative changes in form of diffuse hydropic degeneration of hepatocyte with severe dilatation of the central vein and activation of Von Kupffercell. (H&E, x400)

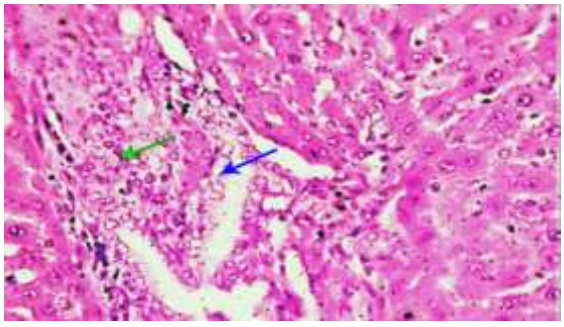

Fig (18) Liver of rat infected with staphylococcus aureus at conc of 10^7 cfu (group3). After seven days from infection, Liver showing mild hyperplastic proliferation of the epithelium lining the bile duct with few periductal leukocytic infiltration.
(H&E, x400).

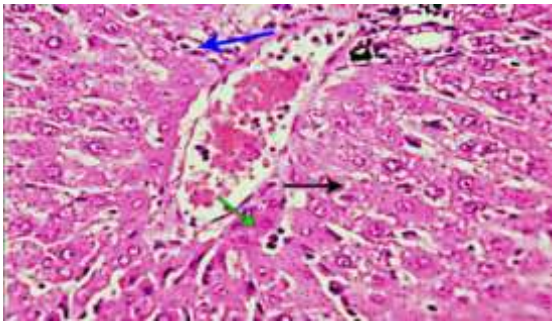

Fig (19) Liver of rats were infected with staphylococcus aureus at conc of 10^7 cfu (colony forming units) followed by administration of moxifloxacin orally for 5 successive days (group4). After seven days from treatment liver showing congestion of central vein and hepatic sinusoid mild degenerative changes and vacuolation in the cytoplasm of hepatocyte.
(H&E,x400)

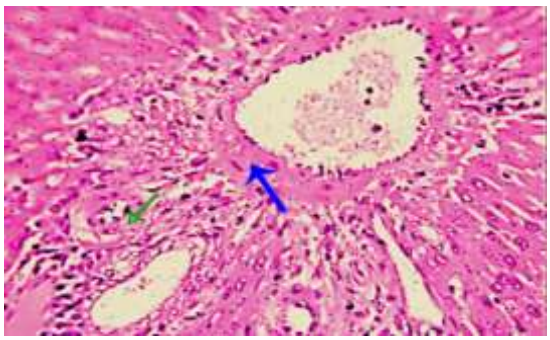

Fig (20) Liver of rats were infected with staphylococcus followed by moxifloxacin treatment for 5 successive days (group4). After 14 day liver showing thickening of the wall of central vein and hyalinization of portal blood vessels with mild intravascular leukocytic infiltration and mild hyperplasia of the lining epithelium of bile duct. (H&E, x400)

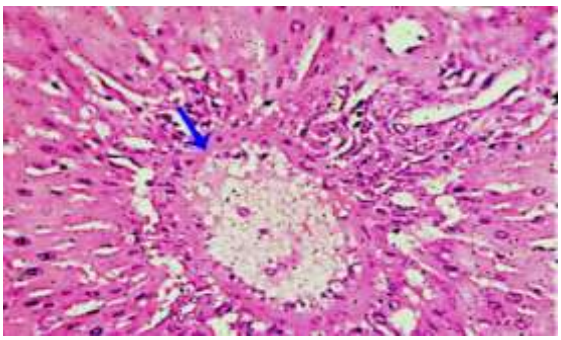

Fig (21) High power of previous figure liver showing thickening of the wall of central vein with mild intravascular leukocytic infiltration. (H&E , x400)

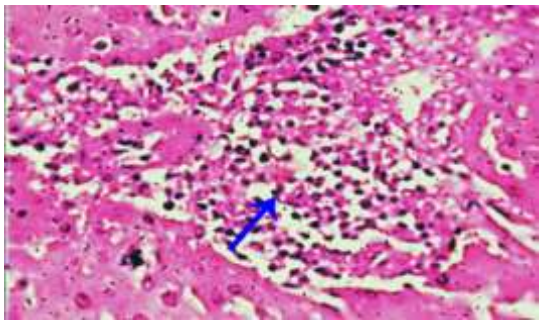

Fig (22) High power of previous figure liver showing focal area of mononuclear cellular infiltration in the hepatic parenchyma (H&E, x400)

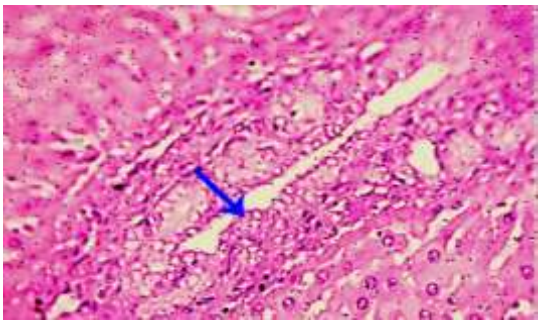

Fig (23) High power of the previous figure. After fourteen day from treatment Liver showing hyperplasia in the epithelial cells lining the bile duct. (H&E , x400**)**

5. Discussion

The present study investigated the adverse effects of therapeutic dose of moxifloxacin 26.4 mg /kg body weight for 5 days in normal, experimentally infected rats with Staphylococcus aureus post treated with moxifloxacin and staphylococcus aureus infected non treated with moxifloxacin .

5.1 Effect of moxifloxacin and or staphylococcus aureus on biochemical parameters

5.1.1 Effect of moxifloxacin and/or staphylococcus aureus on liver function

Liver is considered to be the most sensitive organ in the body. It is the chemical factory because it has three main functions : storage , metabolism and biosynthesis . Glycogen , fat soluble vitamins , fats and other nutrients are stored in liver . It is the main site of xenobiotic enzymes and blood –coagulating factors are synthesized in liver. Occurance of any damage to liver results in cellular damage to liver cells with destruction of their membranes and the releasinge of enzymes into blood stream .

Effect of moxifloxacin ,staphylococcus aureus and staphylococcal infected then treated with moxifloxacin on liver biomarkers as: total bilirubin,aspartatransaminase,alanintransaminstrase, alkaline phosphate, total protein and albumin were investigated .Oral administraion of moxifloxacin for 5 succeccive days at dose of 26.4mg / k.g. of b.wt of rat.Moxifloxacin showed significant increase in serum total bilirubin,aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in rats compared to control group. The fluoroquinolone antibiotics have been reported to generate reactive oxygen species which may result in oxidative stress and cellular damage to the liver and kidney . In this study, moxifloxacin induced marked renal and hepatic damage and oxidative stress and depleted the hepatic antioxidant reserves.

Plasma ALT and AST are reliable marker enzymes of liver function and integrity. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST and ALT are released into the bloodstream. Increase in plasma ALT and AST has been

reported in conditions involving necrosis of hepatocytes, myocardial cells, erythrocyte, and skeletal muscle cells .Our results indicated that treatment of normal rats with moxifloxacin for five days resulted in elevated plasma total bilirubin and activities of ALP, ALT, and AST in experimental animals. Increase in plasma TBILI and ALP activity is known to be associated with hepatobiliary dysfunction which may have resulted from hepatobiliary injury and cholestasis.Data from this study suggested that treatment with moxifloxacin resulted in an increase in plasma total bilirubin and alkaline phosphatase activity caused by moxifloxacin is an indication of hepatobiliary damage. Similar observation was reported by. from studies on a fluoroquinolone antibiotic. Activities of ALT and AST are accepted marker of hepatocellular injury in human and animal models. Elevated plasma alanine aminotransferase and aspartate aminotransferase may be linked with membrane leakage of the hepatocyte cytosolic contents which is reflected in significant elevation of the plasma of rats treated with moxifloxacin or hepatocellular damage as fatty degeneration , fibrosis and impairement of liver functions causing leakage of liver enzymes into the circulation that is normally located in cytoplasm of hepatic cells . Alanine aminotransferase was found in the hepatic parenchymal cells in large amounts and regarded as more liver specific to detect thehepatocellular damage,while aspartate aminotransferase was found in mitochondria particularly in the centerlobular reigon of the liver**.** So , aspartate aminotransferase and alanine aminotransferase are considered the better indicator of any liver damage or injury.

Oral administration of moxifloxacin at 26.4 mg/kg body weight once daily for five successive days in rats.Moxifloxacin showed significant increase in serum total bilirubin,aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in rats compared to control group. Slight increase in serum total protein and serum albumin .

Experimentally infected rats with Staphylococcus aureus showed that infection of normal rats by staph. aureus exhibited a slight increase in serum alanine aminotransferase at the 1st day and 7th day , while aspartate aminotransferase exhibited a non-significant increase at 14th days, when compared with control normal group.While infected rats exhibited a non-significant increase in serum aspartate aminotransferase at the 1st and 14th days, while infected group exhibited a significant increase at the 7th days, when compared with control normal group and showing a non significant increase in serum alkaline phosphatase and serum total bilirubin concentration when compared with control normal group.

On other side experimentally infected rats with Staphylococcus aureus showed a non-significant decrease in serum total protein concentration and serum albumin concentration when compared with control normal group. Staphylococcal infected rats exhibited a non-significant decrease in serum creatine kinase when compared with control normal group.

Post treatment of experimentally infected rats by Staphylococcus aureus with moxifloxacin orally once daily for 5 succeccive days at dose of 26.4mg / k.g. of b.wt of rat exhibited a significant increase in serum alanine aminotransferase , serum aspartate aminotransferase ,alkaline phosphatase, serum total bilirubin concentration and serum total protein when compared with staph. infected none treated group .While the other side of the coin showing a non significant increase in serum albumin when compared with staph. infected none treated group.

5.1.2 Hematological effects

administration of moxifloxacin to normal group of male rats exhibited a significant decrease in blood Hb concentration when compared with control normal group.

Infection of normal rats by staph. aureus exhibited a non-significant decrease in blood Hb concentration while exhibited a significant decrease after 14^{th} days, when compared with control normal group.

Infection of normal rats by staph. aureus exhibited a non-significant decrease in blood Hb concentration at the 1^{st}, 7^{th} days, while exhibited a significant decrease after 14^{th} days, when compared with control normal group.

Administration of moxifloxacin to staph. infected group of male rats exhibited a significant decrease in blood Hb concentration after the 1^{st}, 7^{th}, and 14^{th} days, when compared with staph. infected none treated group.

Administration of moxifloxacin to normal group of male rats exhibited a non-significant decrease in blood RBCs concentration after the 1^{st}, 7^{th} and 14^{th} days, when compared with control normal group.

Infection of normal rats by staph. aureus exhibited a non-significant decrease in blood (red blood cells) RBCs concentration at the 1^{st}, day, while exhibited a non-significant increase after 7^{th} and 14^{th} days, when compared with control normal group.

Administration of moxifloxacin to staph. infected group of male rats exhibited a non-significant decrease in blood (red blood cells) RBCs concentration after the 1^{st}, and 14^{th} days, while exhibited a significant decrease after 7^{th} day, when compared with staph. infected none treated group.

Administration of moxifloxacin to normal group of male rats exhibited a significant decrease in blood (white blood cells)WBCs concentration after the 1^{st}, 7^{th} and 14^{th} days, when compared with control normal group.

Infection of normal rats by staph. aureus exhibited a significant increase in blood (white blood cells) WBCs concentration after the 1^{st}, 7^{th} and 14^{th} days, when compared with control normal group.

Administration of moxifloxacin to staph. infected group of male rats exhibited a significant decrease in blood (white blood cells)WBCs concentration after the 1^{st}, 7^{th}, 14^{th} days, when compared with staph. infected none treated group.

6. Conclusion

In the present work, it was concluded that the effect of therapeutic dose of moxifloxacin (26 mg / kg body weight orally for five successive days) in normal and infected rats with Staphylococcus aureus showed alteration in liver functions .

It would be concluded that, the recorded adverse effects of moxifloxacin, there should be caution with dealing with drug using the different treatments protocols by physicians, assistants and pharmacists. Patient should be monitored and biochemical parameters should be checked before administration of moxifloxacin to avoid the complication during the treatment till recovery.

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