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Some Pharmacodynamic studies on Ampicillin and Enrofloxacin in Male Rats

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Ampicillin, Enrofloxacin, rats, reproductive organs, liver and kidney.

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ABSTRACT

The present study was conducted to evaluate some pharmacodynamic effects of Enrofloxacin and /or Ampicillin each alone and their concomitant administration in male rats. Therefore, sixty mature male albino rats were used and divided into 4 equal groups, each of 15 rats. The first group was kept as a control and rats were subcutaneously injected with distal water at dose of 2 ml./kg. b.wt.

The second group was injected subcutaneously with Ampicillin at a dose of 40 mg/kg. b.wt. twice daily with interval of 6 hours for 5 successive days .

The third group was injected subcutaneously with Enrofloxacin at a dose of 18 mg / kg. b.wt. once daily for 5 successive days .The fourth group was treated with Enrofloxacin (13.5 mg /kg b.w. s/c) once daily concomitantly with Ampicillin (30 mg/kg.b.wt.s.c.) which is repeated after 6 hours from the first dose for 5 successive days.

Five rats from each control and treated groups were killed at 2 nd, 4 th and 8th weeks from the beginning of drug administration.

The obtained results showed that administration of Ampicillin and / or Enerofloxcin each alone and their concomitant administration induced a variety of side effects on male reproduction as reduction of testes, epididymis, and accessory sex organs weights , change in sperm characters, decreased sperm count and motility, and increased the sperm abnormalities. Also, there were some biochemical alterations in the hepatic and renal function. Moreover, these drugs induced some histo-pathological alterations in reproductive organs, liver and kidney.

1. INTRODUCTION

It is well know that the object of all chemotherapy is to eradicate the pathogenic organisms with minimal toxicity to the host. Chemotherapeutic agents should, ideally destroy or immobilize invading organisms and yet leave the host completely unharmed. This will happen if the drugs act on mechanisms that represented in bacteria but not in their mammalian hosts. This has been achieved with many of the anti-microbials because they act on bacterial structures or metabolic functions which are either very different or absent from mammalian cells.

Ampicillin is an antibacterial agent with a broad spectrum of bactericidal activity against both penicillin-susceptible Gram-positive organisms and many common Gram-negative pathogens. It acts by inhibiting the synthesis of bacterial cell wall . Ampicillin is a type of amino-penicillin , a semisynthetic group of β-lactams that were developed for effectiveness against both gram-negative and gram-positive organisms. Amino-penicillins were created by joining penicillin to an amino group or side chain. Addition of the side chain significantly changed the activity of the drug against some bacteria. Initially these antimicrobials were effective against Proteus mirabilis, E. coli, Shigella, Salmonella, Hemophilus and Neisseria species. However due to changes in susceptibility, ampicillin is no longer the drug of choice in treating infections with several of these organisms, such as E. coli

urinary tract infections, unless culture and sensitivity results indicate susceptibility, *(Plumb, 2005)*

Ampicillin stable in presence of gastric acid , when administered parenterally (im, sc) the ampicillin will achieve serum levels of approximately 1/2 of those of comparable dose of sodium salts. Animal studies have demonstrated that ampicillin is distributed throughout the body tissues including liver, lungs ,prostate , muscle , bile and ascites , pleural and synovial fluid .Ampicillin will cross into CSF when meninges are inflamed and only small quantities enter the non- infected cerebro-spinal fluid.Ampicillin crosses the placenta but is thought to be retatively safe to use during pregnancy . Ampicillin is approximately 20%bound to plasma proteins, primarily albumin. Milk levels of ampicillin are considered low. In lactating dairy cattle, milk to plasma ratio is about 0.3.
(Walker, 2007).
Ampicillin is eliminated primarily through renal mechanisms , principally by tubular secretion but some of drug is metabolized by hydrolysis to penicilloic acids (inactive) and then excreted in urine (Plumb, 2005).

Nabata et al. (1988) reported that high doses of the drug or very prolonged use has been associated with neurotoxicity, although the penicillins are not considered hepatotoxic, elevated liver enzymes have been reported.

Enrofloxacine is the first quinolones introduced into veterinary medicine (Walker et al., 2007). All fluoroquinolones are bacteriocidal by inhibiting DNA gyrase (a type II topiosomerase) (Papich and Riviere, 2013). Enrofloxacin is most effective against gram – negative and is indicated for infections of the respiratory , gastro-intestinal and urinary tracts in cattle, pigs and poultry . fluoroquinolones are active against many bacterial types including pseudomonas .These fluoroquinolones show great oral bioavailability, large volume of distribution, low binding to plasma protein that allow them to cross membranes and reach the most remote parts of the body at concentrations above the minimum inhibitory concentration of most pathogens (El-Daly,2013). This property associated with the very wide spectrum of activity make fluoroquinolones first choice for treatment of human, animal and poultry diseases. Fluoroquinolones widely distributed through the body, including the kidneys, liver, bile, prostate, uterus and Fallobian tubes, bones, and inflammatory tissues (Mitchell, 2006). Excretion of thefluoroquinolones is primarily through the kidney , with secondary excretion through the liver (Vancustum, 1990). The adverse effects of the quinolones are limited when compared to their therapeutic efficiency that commonly used in different animals for treatment of different bacterial diseases (Hermo et al., 2010). In food animal species, the liver is considered the primary site of enrofloxacin metabolism in these species, forming ciprofloxacin through oxidative dealkylation. Additional metabolites occurred but comprised less than10 % of the total residue (Plumb, 2005).
The aim of the present study was to evaluate some pharmacodynamic effects and the possible adverse effects of ampicillin and enrofloxacin each alone and their concomitant administration to male rats.

2. MATERIALS AND METHODS

2.1. Drugs

a.**Ampicillin**: (**Epicocillin)** **R**500 mg vial .
manufactured by EIPICO co,Egypt .
b.Enerofloxcin: (Spectramainjectable solution 10%)^{R}.
manufactured by AMON co , Egypt .

Experimental design:

The present study was carried out on 60 mature albino male rats weighting from 130 -170 gm. body weight each and about 4 - 5 months old. Rats were purchased from the Medical Research Institute , Alexandria University The present work was conducted to evaluate the effects of Ampicillin and Enrofloxacin each alone and their concomitant administration in male rats .

The animals were divided equally into 4 groups each of 15 rats as follows:

Group I: Rats were injected with distilled water at a dose of 2 ml./kg. b.w. s/c for 5 days and kept as a control group .

Group II: Rats were injected with ampicillin (40 mg./ kg. b.w. s/c)twice daily with interval of 6 hours for 5 successive days .

Group III: Rats were injected with enrofloxacin(18 mg. / kg. b.w.s/c) once daily for 5 successive days .

Group IV: rats were injected once daily with enrofloxacin (13.5 mg./kg.b.w . s/c) concomitantly with ampicillin at a dose of 30 mg/kg. b.w. s/c . Ampicillin injection is repeated after 6 hoursfor 5 successive days.

The doses of Enerofloxcin and Amplicillinwere calculated according to the method described by *Paget and* Barnes (1964) and Awobajo (2010) respectively.

Five rats from each treated and control groups were killed after 2nd, 4th, 8th week from the beginning of drug administration. Blood,some body organs and epididymal contents were obtained from treated and control rats for examination and evaluation .

Blood sampling:

Two blood samples from each control and treated rats were taken before sacrifying them from orbital plexus (inner canthus of the eye) under light ether anaesthesia using heparinized hematocrite tube. One sample was taken with EDTA for blood picture while the other sample was taken without anticoagulant and left to clot at room temperature then centrifuged for 15 min at 3000 r.p.m to obtain clear serum. The sera were identified and stored in deep freezer at −20 ºC till used for biochemical analysis.

I- Haematological studies:

Thehaematological parameters were measured automatically by using the haematology Analyzer device which give a full report for complete blood picture as (Blood Analysis Mindray BC-30S Auto CBC Blood Hematology Analyzer Device).

II- Biochemical studies:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was activities were measured colourimetrically according to the method described by Reitman and Frankel (1957) and Alkaline phosphatase activity was measured according to the method described by Kind and King (1954). Serum urea activity was measured by the enzymatic colourimetric method as described by Coulomb and Farreau (1963).Serum creatinine activity was measured by the colourimetric kinetic method as described by Husdan and Rapoport (1968).

III- Fertility studies:

Rats were sacrifized by decapitation and the epididymal content of each rat was taken by sharp cutting of the tail of epididymes and squeezed gently on sterile glass watch to estimate the progressive motility, sperm cell count and sperm abnormalities according to the method described by Berdan and Fuquay (1980).

a- Sperm progressive motility and abnormalities:

A clean dry slide was placed on heated stage microscope and allowed to warm. A drop of semen was placed on the clean dry slide, mixed with two drops of saline using glass rod. Uniform mixture must be prepared to estimate accurate determination. . Three hundred sperms were observed under high power lens and the percentage of abnormal sperms was estimated and recorded.

b-Epididymal sperm count:

For counting epidiymal sperm, a hemocytometer and a pipette were used. A drop of cauda epididymal content of each control and treated rats was withdrawn up to mark 0.1 and the pipette was then filled up to the mark 101 by the sodium bicarbonate solution 5% for breaking up the mucus droplets in the hemocytometerpipette .The sperm cell count was achieved microscopically by using high power .

IV- Weight of Internal Body Organs:

After collection of the blood samples and epididymal sperm examination, testes , accessory sex organs (prostate and seminal vesicle) ,epididymis , were dissected out , grossly examined and weighed . The index weight [I.W] of each organ was calculated as described by Matousek (1969).

$$\text{Index weight[I.W.]} = \frac{\text{organ weight}}{\text{Body weight}} \times 100$$

V- Histopathlogical studies:

Following complete necropsy of the experimental male rats, small fresh specimens from liver, kidney, testes, epididymis, accessory sex organs were collected and rapidly fixed in 10 % formalin solution for at least 24hr .Afterthat ,these specimens were processed through the convential paraffin embedding technique [dehydration in ascending grades of ethyl alcohol , clearing in different changes of xylene and embedding in different changes of melted paraffin wax at 60c].paraffin blocks were cut by microtome into 5 microns ,thick sections which were stained by Haematoxylin and Eosin [H.E] ,according to the method described by Harries (1989) and were examined.

VI - Statistical analysis

Statistical analysis was performed using the SAS computer program (SAS, 2002).

3. RESULTS

A – Haematological Findings :

There was a significant decrease in R.B.Cs count in groups treated with Enerofloxacin and Ampicillin + Enerofloxcinat the 4thand 8thweek of the experiment as compared with control group but there was decrease in group treated with Ampicillin at 4thweek of the experiment as compared with control group and there was a reduction in WBCs count in all treated groups

at 4th week of the experiment and in Ampicillin treated group at 2nd week of the experiment as compared with control group .Also, there was a decrease in HB value in group treated with Ampicillin + Enerofloxcin at 8th week as compared with control group .Moreover there was a reduction in PCV value in all treated groups at allover experimental periods as compared with control . (Table 1 and2).

B- Biochemical Findings:

The obtained data showed that there was a significant increase in serum ALT & AST values in group treated with Enerofloxcin at 4th weeks of the experiment as compared with the control group. There were increase of ALP level in all treated groups at 4th and 8th week as compared with control group. (Table 3) .

Also, there was a significant increase serum urea in all treated groups at the 8th week of the experiment and there was elevation in creatinine level in all treated groups at allover experimental periods as compared with control (Table 4).

C- Reproductive Findings:

1-Reproductive organs index weight:

The obtained results showed that there was decrease in the index weight of testis of mature male rats treated with Ampicillin at 8th week from injection as compared with control group. Also a significant increase in index weight of testis in all treated groups was observed after 4th weeks from drug administration as compared with control . (Table 5)

The obtained results showed that there was a significant decrease in the index weight of seminal vesicle of mature rats in all treated groups at all experimental periods as compared with control group . Aso, there was significant decrease in index weight of epididymis of mature rats in all treated groups at 2nd week from drug administration as compared with control. (Table 5)

2-Sperm motility%, sperm count, sperm abnormalities%:-

The obtained results showed that subcutaneous administration of Ampicillin and its concomitant administration with enerofloxacin induced a significant decrease in the progressive sperm motility and sperm count values at 2nd week of the experiment, while there was a significant decrease in their values in all treated groups at 4th , 8th weeks from drug administration as compared with control group. However**,** there was a significant increase in sperm abnormalities in all treated groups at the 2nd week of the experiment as compared with control group (Table 6)

Table 1:. Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administration RBCs and WBCs counts in adult male rat at different periods from beginning of drugs administration .

Parameters	**RBCs count (×106/ cmm)**			**WBCs count (×103 /cmm)**		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	Bc	Aa	Ab	Ab	Aa	Bc
	0.48±3.33	0.43±4.86	1.82±4.6	1.00±7.73	0.35±8.45	1.39 ±7.10
Ampcillin	ABb	Cc	Aa	Bc	Bb	Ba
	0.61±3.44	0.84±2.92	1.77±4.72	0.97±5.10	1.32±7.98	2.74 ±7.13
Enerofloxcin	Bb	Ba	Cc	Aa	Cb	Aa
	o.99±3.05	1.29±4.45	0.77±2.84	1.71±7.05	1.03±6.90	4.69 ±8.84
Ampcillin	Ca	Cb	Ba	Aa	Cb	Aa
+Enerofloxcin	0.36±2.53	0.76±2.88	1.45±3.87	0.78±7.35	1.76±6.70	1.77 ±8.42

- Capital letters: Means within the same column of different letters are significantly different ($p<0.05$).
- Small letters: Means within the same raw of different letters are significantly different ($p<0.05$).

Values are expressed as Means±S.E n=5

Table 2:. Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administration HB g % and PCV % and in adult male rat at different periods from beginning of drugs administration .

Parameters	**HB (g/dl)**			**PCV%**		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	Ac	Aa	Bc	Ab	Aa	Ab
	14.75±1.26	0.60±14.40	14.90±2.25	37.58±3.48	38.30±2.80	38.33±8.94
Ampcillin	Ac	Ab	Aa	Bb	Cc	Ba
	15.10±1.61	0.93±15.23	17.97±0.33	28.23±3.94	26.40±4.72	30.53±6.77
Enerofloxcin	Aa	Ab	Bc	Cb	Ba	Cc
	15.00±0.60	2.65±15.50	15.67±3.50	21.83±3.62	36.18±10.35	20.93±6.41
Ampcillin	Ab	Bc	Aa	Ca	Cb	Ba
+Enerofloxcin	14.80±1.33	2.33±13.53	17.12±3.35	23.40±3.13	27.28±6.54	24.28±8.91

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Values are expressed as Means±S.E n=5

Table 3: Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administration on liver enzymes levels in adult male rat at different periods from beginning of drugs administration .

Parameters	**ALT(U/L)**			**AST(U/L)**			**ALP (U/L)**		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	Ba	Bb	Ac	Ba	Bb	Ac	Aa	Db	Dc
	36.60±1.96	38.20±1.07	27.33±1.76	45.20±9.52	43.60±1.86	46.00±3.06	144.36±18.81	145.60±13.	141.00±0.58
Ampcillin	Aa	Bb	Ac	Ba	Bb	Ac	Ac	Cb	Aa
	44.50±6.80	40.00±1.41	22.65±3.27	48.75±26.0	41.25±1.18	45.75±3.35	144.10±11.56	160.00±18.	270.25±39.1
Enerofloxcin	Bb	Aa	Ab	Ba	Aa	Ab	Ac	Ba	Cb
	31.50±4.11	48.75±1.11	30.50±1.26	51.13±2.68	51.00±2.55	40.00±1.22	132.85±7.58	261.75±21.	198.75±6.24
Ampcillin	Bb	Aba	Ac	Aa	Bb	Ac	Ac	Aa	Bb
+Enerofloxcin	38.00±1.35	42.75±1.38	25.75±5.02	59.33±4.95	46.25±5.51	51.50±0.96	121.83±6.24	321.00±27.	260.50±30.2

Values are expressed as Means±S.E n=5

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Table 4: Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administration on Urea level (mg/dl) and Creatinine level (mg / dl) in adult male rat at different periods from beginning of drugs administration .

Parameters	**Urea (mg /dl)**			**Creatinine (mg/ dl)**		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	Ab	Aa	Bc	Cc	Ca	Cb
	22.33±3.46	24.21±0.34	21.61±2.55	0.36±0.08	0.38±0.12	0.35±0.05
Ampcillin	Ab	Aa	Ac	Ab	Bc	Ba
	22.62±1.71	24.96±1.95	29.25±2.02	1.17±0.10	0.96±0.05	1.29±0.23
Enerofloxcin	Aa	Aa	Ac	Bc	Ab	Aa
	22.84±0.93	24.62±1.26	28.13±1.56	0.70±0.15	1.60±0.16	1.59±0.44
Ampcillin	Aa	Ab	Ac	Bc	Ab	Aa
+Enerofloxcin	24.15±0.26	24.19±0.99	33.70±5.38	0.71±0.13	1.65±0.13	1.50±0.13

Values are expressed as Means±S.E n=5

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Table 5:. Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administration on the index weight of reproductive organs in adult male rat at different periods from beginning of drugs administration .

Parameters	Testes weight index			Seminal vesicle index weight			Epididymis index weight		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	1.00±0.12 Ba	1.04±0.15 Ca	1.01±0.07 Ba	0.92±0.04 Aa	0.72±0.02 Ab	0.89±0.06 Ac	0.49±0.04 Aa	0.48±0.05 Ab	0.48±0.04 Aa
Ampicillin	1.03±0.04 Bb	1.15±0.07 Ba	0.93±0.08 Cc	0.70±0.09 Ca	0.49±0.05 Bb	0.41±0.05 Cc	0.40±0.01 Bc	0.48±0.03 Aa	0.43±0.02 Ab
Enerofloxcin	1.18±0.05 Aa	1.16±0.10 Bb	1.02±0.09 Bc	0.52±0.06 Da	0.35±0.03 Cb	0.23±0.06 Dc	0.40±0.03 Bc	0.48±0.03 Aa	0.44±0.05 Ab
Interaction	1.16±0.06 Ab	1.31±0.10 Aa	1.11±0.05 ABc	0.78±0.09 Bb	0.48±0.03 Bc	0.60±0.05 Ba	0.37±0.03 Cc	0.51±0.04 Aa	0.47±0.04 Ab

Values are expressed as Means±S.E n=5
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Table 6:. Effect of subcutaneous administration of Ampicillin (40 mg/kg bwt) or Enerofloxcin(18 mg/kg b.w) and their concomitant administrationon fertility parameters in adult male rat at different periods from beginning of drugs administration .

Parameters	Sperm motility (%)			Sperm count (x106/ml)			Sperm abnormalities (%)		
Time Group	2nd week	4th week	8th week	2nd week	4th week	8th week	2nd week	4th week	8th week
Control	86.25±2.39 Ab	95.00±0.01 Aa	86.67±1.6 Ab	544.44±42.48 Aa	565±135 Ab	550±18.93 Ac	19.80±2.48 Db	18.00±1.00 Ac	25.67±0.67 Aa
Ampicillin	72.00±2 Bb	83.00±3.74 Ba	74.00±2.45 Cb	435±23.29 Bb	317±54.19 Dc	446.20±91.7 Ba	33.20±1.88 Ba	17.50±1.64 Ac	23.80±2.59 Ab
Enerofloxcin	84.00±2.92 Aa	81.00±2.45 Ba	73.00±2 Cb	549±48.72 Ab	408±59.15 Ba	307±114.88 Dc	39.20±3.79 Aa	19.10±1.11 Ac	25.60±0.99 Ab
Interaction	71.00±1 Bb	78.75±1.25 Ca	76.00±2.9 Ba	422±60.92 Ca	385±33.73 Cb	421.20±6.4 Ca	28.20±2.67 Ca	18.38±1.39 Ac	25.50±2.05 Ab

Values are expressed as Means±S.E n=5
- Capital letters: Means within the same column of different letters are significantly different ($p<0.05$).
- Small letters: Means within the same raw of different letters are significantly different ($p<0.05$).

D-Histopathological Findings :

Histological examination of some body organs of male rats treated with Ampicillin or Enrofloxacin each alone and their concomitant administration were carried out at 2 ,4 and 8 weeks from the beginning of drug administration.

(A) Macroscopic Findings:

Macroscopic examination of the organs of male rats treated with Ampicillin or Enrofloxacin each alone and their concomitant administration revealed no visible pathological alterations of the liver, kidney , and the reproductive organs of treated male rats as compared with the control group .

(B) Microscopic Findings:

The testicular tissue of male rats treated with Ampicillin or Enrofloxacin each alone and their concomitant administration showed congestion of the interstitial blood vessel, hypo- spermatogenesis, interstitial edema at the 4th and 8 th the weeks of the experiment (Fig1, Fig 2, Fig 3).

The microscopical examination of the livers in all treated rats showed severe hydropic degeneration,vacuolation and congested portal blood vessels with few lymphocytic infiltrations , congestion and lymphocytic reaction at the 4th and 8th the week of the experiment (Fig. 4. Fig . 5).

The examined kidneys of rats treated with Ampicillin or Enrofloxacin and their concomitant administration revealed congestion (cortical or medullary),hemorrhages, lymphocytic infiltration, tubular dilation and tubular cast and excess of luminal casts in the cortical tubule at the 4th and the 8th week of the experiment (Fig. 6 , Fig . 7)

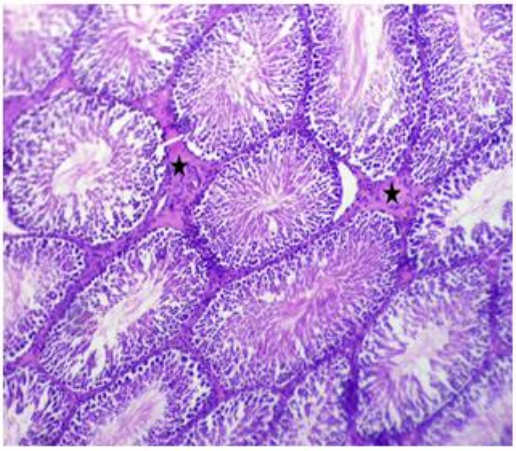

Fig. (1): Testis of a rat of treated with Ampicillin together with Enerofloxcin at 4th week from drug administration showing congestion of interstitial blood vessel (stars) . H&E. (x160).

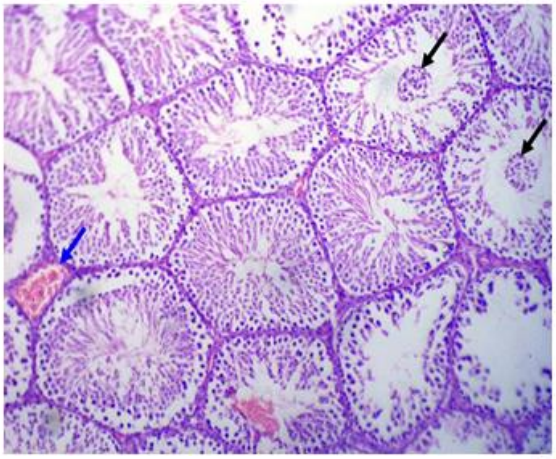

Fig. (2): Testis of a rat treated with Enrofloxcinat 8th week from drug administration showing congestion of interstitial blood vessel and sloughing of the germinal epithelium in the lumen of the seminepherous tubules (arrows). H&E. (x160).

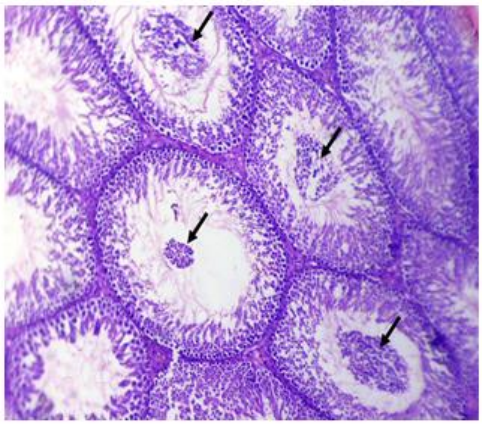

Fig. (3): Testis of a rat treated with ampicillin at 4th week from drug administration showing sloughing of the germinal epithelium in the lumen of the seminepherous tubules (arrows). H&E. (x160).

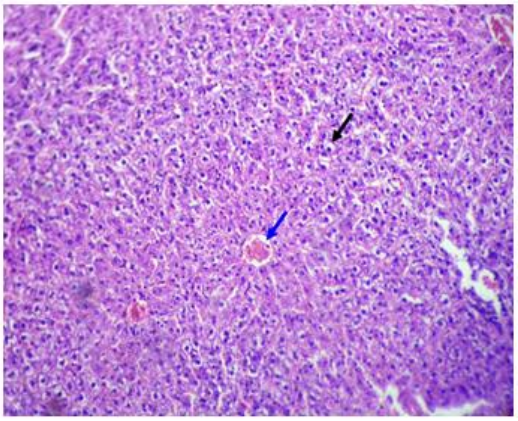

Fig. (4): Liver of a rat treated with Enrofloxcin at 4th week from drug adminstration showing congestion of blood vessel (arrow) and hydropic degeneration of the hepatocytes . H&E. (x160).

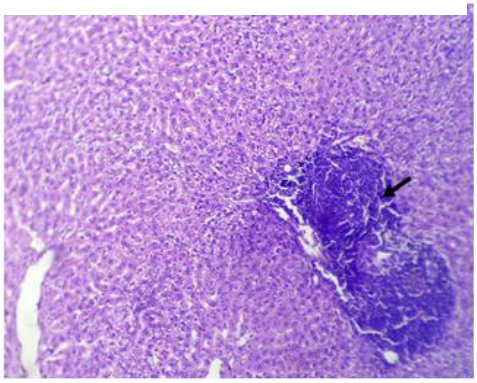

Fig. (5): Liver of a rat treated with Ampicillin together with Enerofloxcin at 8th week from drug administration showing intense inflammatory cells infiltration in the portal area (arrow). H&E. (x160).

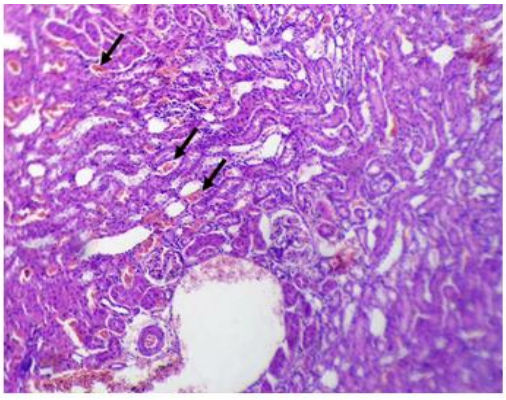

Fig. (6): Kidney of a rat treated with Enrofloxcinat 8th week from drug administration showing severe congestion of intertubular blood capillaries (arrows). H&E. (x160).

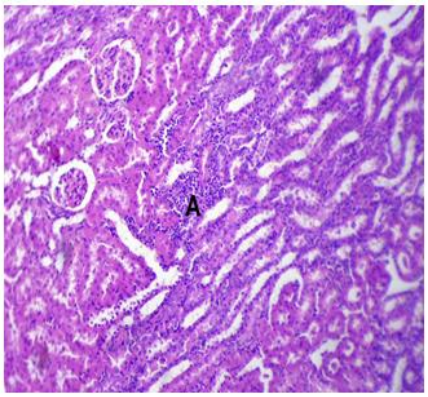

Fig. (7): Kidney of a rat treated with Ampicillin at 4th week from drug administration showing interstitial nephritis where inflammatory cells infiltration in the interstituim (A). H&E. (x160).

4. DISCUSSION

The present study was carried out to evaluate some pharmacodynamic properties and the possible adverse effects of Enrofloxacin and Ampicillin each alone and their concomitant administration to male albino rats . Haematological , biochemical and histo-pathological investigations were carried out at 2, 4 and 8 weeks from onset of drug administration to follow up the induced effects. The duration of the present study lasts for two months to cover complete spermatogenic cycle in rats which range from 48 – 52 days (Clermont and Harvey, 1965).

The results of the effects of different treatments on R.B.Cs. count cleared that, a significant decrease in R.B.Cs count in groups treated with Enerofloxacin and Ampicillin + Enerofloxcinat the $4^{th}$and 8^{th}week of the experiment but there was decrease in group treated with Ampicillin at 4^{th}week of the experiment and there was a reduction in WBCs count in all treated groups at 4^{th}week of the experiment and there was a decrease in HB value in group treated with Ampicillin + Enerofloxcin at 8^{th} week .Moreover there was a reduction in PCV value in all treated groups at all over experimental periods as compared with control .The present results are incompatible with those reported by Al- Mayah and Al-Ahmed (2005), they found that, oral administration of enrofloxacin for 5 consecutive days induced changes in PCV, Hb, RBCs, MCV, MCH, MCHC and HGB. The investigations demonstrated a fall in hematological values in young chicks, whereas in older ages these values were analogus with normal range. The changes were not substantiate the presence of anemia. They might be indications of an incidental haemodilution.

The obtained results are confirmed by Tars et al. (2001). who reported, that intramuscular administration of enrofloxacin 5 mg/Kg, once daily to 10 healthy dogs for 14 days caused acidosis and temporary increase in aspartate aminotransferase. On the other hand, the serum level of urea significantly increased after 2 weeks from enrofloxacin administration at both doses (30 and 60 mg/Kg B.Wt). While, significant increase in creatinine level all over the period of experiment at the two dose levels. Transaminases including AST and ALT are found in most tissues but in unequal proportions, ALT occur exclusively in the liver, but only in the cytoplasm of paenchymal cells, in contrast to AST which is equally distributed between the cytoplasm and mitochondria. Doxy (1971) mentioned that the level of these enzymes is increased following liver damage. Accordingly the obtained results of elevation of ALT and AST levels after enrofloxacin administration are attributed to damage of the hepatic cells by the direct effect of the drug and/or its metabolite resulting in escape of these enzymes to plasma.

Our results showed a significant increase of serum urea in all treated groups at the 8^{th} week of the experiment and there was an increase in creatinine level in all treated groups at all over experimental period as compared with control.

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These results also were confirmed by our histopathological finding in hepatic tissue as there was severe hydropic degeneration, vacuolation and congested portal blood vessels with few lymphocytic infiltrations , congestion and lymphocytic reaction .

Our results on the effects of different treatments on internal organs weight of male rats, that there was decrease in the index weight of testis of mature male rats treated with Ampicillin at 8th week from injection as compared with control group.

The obtained results showed that there was a significant decrease in the index weight of seminal vesicle of mature rats in all treated groups at all experimental periods as compared with control.

The results of organ weight from rats treated orally with

therapeutic doses of the ampicillin, cloxacillin, and tetracycline for fourteen days showed that there signifi cant ($P<0.05$) reduction in epididymal weight in ampicillin and cloxacillin treated groups where degenerative changes in the testicular histology were found (Nwunuji, 2015).

Our results of reduction of weight of sexual organs, sperm motility and count in ampicillin treated group may be attributed to, the decreased fructose and protein level that affect glycoproteins secreted by the epididymis and coated on the sperm to stimulate motility (Gupta et al., 2013). The reduced protein content may be another reason for the reduction in the weight of reproductive organs, because the growth rate of organ is proportional to its protein content (Sarkar et al., 2000).

Also, the results of this study are in agreement with Aral et al.(2008) who suggest that administration of enrofloxacin subcutaneously to male mice at a fixed dose of 150 mg/Kg once daily for 15 days, would lead to disruption of spermatogenesis in the testes causing deterioration of motility and content of sperms as well as morphological abnormalities.

These results were confirmed by histopathological finding in renal tissue of treated rats as there was congestion, hemorrhages, lymphocytic infiltration, tubular dilation and tubular cast and excess of luminal casts in the cortical tubule.

This suggestion is supported by the reported histopathological alterations of the present work which revealed congestion of the interstitial blood vessel, hypo- spermatogenesis, and interstitial edema.

These results were confirmed by histopathological finding in renal tissue of treated rats as there was congestion, hemorrhages, lymphocytic infiltration, tubular dilation and tubular cast and excess of luminal casts in the cortical tubule.

CONCLUSION

The administration of Enrofloxacin (18 mg/kg b.wt. s/c) or Ampicillin (30 mg/kg b.wt. s/c) each alone and their concomitant administration to mature male rats induced a variety of adverse effects. These are represented by some fertility troubles such as decreased sperm motility and count and an increase in total sperm abnormalities with some histopathological alterations in the reproductive organs. These drugs induced some degrees of hepatic and renal damage.

It could be concluded that administration of Enerofloxcin or Ampicillin each alone and their concomitant administration to mature male rats induced some adverse effects on liver, kidney and the reproductive organs of male rats.

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