Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 5
ISSN:2321–3647(online)

Regardless of the Antibiotic Group or Type, Misuse of antibiotics Adversely Impair Liver, Kidneys and Heart Functions "Biochemical and Histopathological Assessment"

Wud M. E. Siddig[1] Mohammed A. A. Salim[2], Magdi Badawi[3], Abdelgadir B. Mohamed[3], Ishraga G. Ibrahim[3], Mona K. Farid[4] and Emad H. E. Konozy [5*].

1.Department of Zoology, University of Khartoum, Khartoum, Sudan
2. Biotechnology Park, Africa City of Technology, Khartoum, Sudan
3.Veterinary Research Institute, Madani St, Khartoum, Sudan
4. Department of Pathology, Faculty of Basic Medical Sciences, Libyan International Medical University, Benghazi, Libya
5. Department of Biochemistry, Faculty of Basic Medical Sciences, Libyan International Medical University, Benghazi, Libya

ABSTRACT

This study was conducted in 78 roosters *Hisex white layer* to investigate the possible effect of use or misuse of antibiotics on liver and kidneys function as well as some other important organs. Three antibiotics of varying groups i.e., tylosin, ciprofloxacin and oxytetracycline were chosen for the study. Antibiotics were daily orally administered in different doses as described in the experimental body. The possible adverse effect of antibiotics on liver and kidneys functions were followed by testing liver and kidneys serum biomarkers such as Glutamate oxido transaminase (GOT), Glutamate pyruvate transaminase (GPT), acid phosphatase (ACPase), alkaline phosphatase (ALKPase), total protein, albumin, globulin etc. Antibiotics residues clearance in plasma samples were also studied and correlated to administered dose. Histopathological investigations were further supported our biochemical data on the adverse effect of antibiotics on kidney, liver, heart, and other tissues. The present study clearly concluded a direct adverse effect of antibiotics, regardless of the chosen group, on liver and kidney and heart function.

Keywords: Antibiotics; misuse; chicken; Biochemical analysis; liver; kidney; heart

*Corresponding Author Email: ehkonozy@yahoo.com
Received 11 April 2014, Accepted 17 May 2014

Please cite this article in press as: Emad HEK *et. al.*, Regardless of the Antibiotic Group or Type, Misuse of antibiotics Adversely Impair Liver, Kidneys and Heart Functions "Biochemical and Histopathological Assessment". American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Antibiotic is a compound or substance that kills or fight bacterial growth. Antibiotics are broadly classified based on their structure, mechanism of action and or range of mechanism of action, in which most of them work on arresting bacterial growth and finally their death[1].

Some antibiotic target cell wall (penicillin and cephalosporin), or the cell membrane (polymixins), while others could interfere with metabolically important bacterial enzymes (quinolones and sulfonamides). Of great important is the class of antibiotics which interferes with machineries of protein synthesis in bacteria [2].

Antibiotics are dispensed to animals for a number of different reasons: therapeutic treatment, disease prophylaxis and growth promotion. In poultry antibiotics are used also for growth promotion. Edible tissues containing veterinary drug residue in tissues and food of animal which detected using HPLC technique [3], can pose risks to human health including: direct toxic effect- allergic reactions- increased bacterial resistance to common antibiotics, which occurs when strains of bacteria in the body become resistant to antibiotic, it's a worldwide public health problem [4].

The most common misuse of antibiotics are prescription of antibiotic for viral infection, self medication without a doctor referral[5, 6] and the full dosage of antibiotic is not finishing. The hazards of antibiotic misuse may prove to be life threatening by accelerating bacterial resistance antibiotics. Antibiotic resistance has led bacteria to changing their structure, so antibiotic no longer works. Inactivate or neutralize the antibiotic .Transfer the gene coding between them [7].

The important organs which will be affected by abuse of antibiotic is a liver and Kidney. Liver manufacture their own structural proteins and intracellular enzymes [7]. Liver enzymes are proteins that help to speed up a chemical reaction in the liver, under normal circumstances these enzymes residue within the cells of the liver, but when the liver is injured or damaged, enzymes are spilled into the blood [7]. In the same manner several biomarkers are routinely used to follow damage or renal diseases [8, 9].

The phosphatases are enzymes responsible for many functions in the body metabolism, absorption of food from the gastrointestinal tract, dephosphorylation within the liver, re-absorption of glucose by the renal tubules, deposition of lime salts within the bones. Phosphatase enzymes include acid phosphatase which present in highly level in prostate, stomach, liver, spleen and erythrocytes, alkaline phosphatase present in highly level in bone, liver, placenta, kidney, intestine ,used to measure of the secretary function of the liver [10].

Albumin is made and excreted via the liver, used as marker for the synthetic function of the liver. It is essential crucial in maintaining body's osmotic pressure, assists in transportation of hydrophobic essential nutrients and minerals as well as its function as a storage protein and to act as a direct source of amino acids whenever needed [11].

Total Protein made and excreted via the liver, used as marker for the synthetic function of the liver, play roles in the maintenance of colloid osmotic pressure, in transport of minerals, hormones, in build of enzymes, immune system in the organism [11].

Amino transferases are a group of enzymes that catalyze the interconversion of amino acids and oxoacids by transfer of amino groups, includes aspartate aminotransferase is found in highest concentration in heart, muscle, liver, and alanine aminotransferase which exclusively found in highest concentration in liver [12].

The prevalence of self-medication with antibiotics in Sudan is alarmingly high [6]. These misuses range from repeated administration of antibiotic without a doctor consultancy and sometimes with no clear information on doses. This study was therefore undertaken to explore on the hidden side effects of the misuse or abuse of antibiotics on chicken model.

MATERIALS AND METHODS

Seventy eight, three months old *Hisex White Layers* male chickens (roosters), were generously donated by *Coral Agricultural and Animal Production Company*, Khartoum, Sudan. Animals were raised in the animal house of the institute of veterinary research, Sobba, Khartoum South. Fed on standard poultry formulated feed and provided with drinking water.

Antibiotics

All antibiotics were purchased from local pharmacies. Used antibiotic were as follows: Oxytetracycline was manufactured by shanghai pharmaceuticals industrial Company, China, whereas Ciprofloxacin was from *Ibn hayyan* pharmaceticals company, Syria. On the other hand, tylosin was obtained from General Medicines Company, Sudan.

Antibiotic Administration

Antibiotics were administered parenteral according to animal respective weight as given below.

Doses

Administered antibiotics were classified according to doses and duration in the following manner:

Ciprofloxacin [13, 14]

Normal dose for normal duration (given for 5 days): 8mg/kg/day

Over dose for normal duration (given for 5 days) : 16mg/kg/day

Normal dose for long duration (given for 7 days) : 8mg/kg/day

Over dose for long duration (given for 7 days) : 16mg/kg/day

Tylosin[15]

Normal dose for normal duration (given for 5 days): 8mg/kg/day

Over dose for normal duration (given for 5 days) : 16mg/kg/day

Normal dose for long duration (given for 7 days) : 8mg/kg/day

Over dose for long duration (given for 7days) : 16mg/kg/day

Oxytetracycline[16]

Normal dose for normal duration (given for 5 days): 10mg/kg /day

Over dose for normal duration (given for 5 days) : 20mg/kg /day

Normal dose for long duration (given for 7 days) : 10mg/kg/day

Over dose for long duration (given for 7days) : 20mg/Kg/day

Experimental design

The birds were divided into four groups; each group consisted of 24 roosters, besides a control group consisted of 6 birds. This classification is done based on planned doses and type of antibiotics used in the study.

Samples Collection

Three mL blood samples were withdrawn on 2^{nd}, 4^{th}, 6^{th}, 8^{th} day for tylosin and ciprofloxacin while on 2^{nd}, 4^{th}, 8^{th}, 10^{th} day for oxytetracycline from jugular vein of each rooster. Samples were collected in either plain or heparinised tubes. Plasma and serum were separated by centrifugation at 3000 *rpm* for 10 min and stored frozen at – 20ºC until further use.

Biomarkers analysis

Acid phosphatase (ACPase)[17]

This enzyme was estimated using Spinreact kit (Crta. Sta. Coloma, 717176 St. Esteve d'en Bas, GIRONA – Spain) according to manufacturer's instructions.

In brief: 0.05 mL of the enzyme (serum) was incubated with 0.5 mL of pseudosubstrate α Naftyl phosphate -Fast Red *TR* under standard assay conditions of temperature and pH. Liberated color of α-naphtol reacted with diazoted compound was monitored spectrophotometrically at 405nm.

1 Unit of enzyme is defined as amount of enzyme that transforms 1µmol of substrate min/L under standard conditions.

Alkaline phosphatase (ALKPase)[18]

The Alkaline phosphatase was estimated using Spinreact kit (Crta. Sta. Coloma, 717176 St. Esteve d'en Bas, GIRONA – Spain) according to manufacturer's instructions. In brief: 0.01mL of the enzyme (serum) was incubated with 0.6 ml of substrate ρ-*nitrophenyl phosphate* under standard assay conditions of temperature and pH. Liberated color of ρ-nitrophenol was monitored spectrophotometrically at 405nm.

Aspartate aminotransferase (AST)[19]

This enzyme was estimated using Spinreact kit (Crta. Sta. Coloma, 717176 St. Esteve d'en Bas, GIRONA – Spain) according to manufacturer's instructions**.** In brief: To 0.5mL of substrate α-ketoglutarate 0.1 mL of serum was added under standard assay conditions of temperature and pH**.** The oxidation rate of NADH to NAD+ was monitored spectrophotometrically at 340nm.

Alanine aminotransferase (ALT)[19]

This enzyme was estimated using Spinreact kit (Crta. Sta. Coloma, 717176 St. Esteve d'en Bas, GIRONA – Spain) according to manufacturer's instructions. In brief: 0.1 mL of serum was incubated with 0.5 mL of substrate α –ketoglutarate under the standard assay conditions of temperature and pH, the oxidation rate of NADH to NAD+ is monitored spectrophotometrically at 340nm.

Total protein Estimation

This was done according to the buiret protein estimation method [20]. In short, the assay was performed by mixing diluted serum sample to which 5mL of freshly prepared buiret reagent was added, mixed well and heated gently at water bath at 37°C for 10min. Developed blue color was measured spectrophotomertically at 540nm.

Albumin estimation by bromocresol green

Bromocresol green binds quantitatively to albumin forming an intense blue-green complex with an absorbance maximum at 628 nm. The intensity of the developed color is directly proportional to the albumin concentration in the sample [21].

Plasma Residual Concentrations of tylosin

This was measured by microbiological assay for tylosin detection according to van leeuwen method, in brief: To 1.4g of nutrient agar 50 mL of distilled water was added, autoclaved at 121°C for 1hour. The molten agar was left at room temperature to warm, then 0.2g of dextrose were added followed by 2µL of *Bacillus subtilis* under sterilized conditions. After incubation the inhibition zone was monitored [22] .

Statistical analysis

Statistical analysis was done using the statistical software SPSS version 12. 2 samples T. test (independent T. test) used to compare treated groups with their untreated group (control). P. value <0.05 was considered as statistically significant for the purpose of this study.

Preparation of tissues

Tissue Harvesting: animal experiments were conducted in the institute of veterinary medicine, Khartoum, Souba, according to Institutional Animal Care and Use Committee standards. Roasters were sacrificed for use in the development and validation of these processing methods. Tissues were collected from different organs as stated. The obtained tissues were immerged into 2% para formaldehyde and 2% glutaraldehyde fixative.

Paraffin Processing for Light Microscopy

Tissues from varying organs were fixed in 10% neutral buffered formalin for 2 days, dehydrated in alcohol, cleared with xylenes, infiltrated, and embedded into Paraplast X-TRA paraffin on a Tissue-Tek VIP tissue processor. Five-micrometer sections were cut with a microtome and placed onto Superfrost Plus Slides and stained with a routine H&E method as described below.

H&E Staining of Paraffin Sections

H and E staining was done as described. Following a standard deparaffinization, slides were stained in Hematoxylin 1 for 10 min followed by a running water wash. After 1 min each in Clarifier 2 and Bluing solution (Richard-Allan Scientific), the slides with mounted tissues were stained in Eosin Y with Phloxine for 2 min, dehydrated, cleared and mounted with TBS SHUR/Mount Xylene-Based Mounting Medium. Slides were then cover slipped as shown [23, 24]

RESULTS AND DISCUSSION

The results of administration of different doses of antibiotics (ciprofloxacin, tylosin and oxytetracycline) at different durations on chicken's liver and kidneys function are shown in figures 1, 2 and 3 as well as Tables 1 and 2.

Table 1: Effect of tylosine on biomarker of liver and kidney function

Test	Control	Normal dose Short time	Over dose Short time	Normal dose Long time	Over dose Long time
ALKPase (U/L)	60.10± 17.45	106.48± 24.81	41.46± 13.79	139.92± 33.90	88.44± 32.29
ACPase (U/L)	3.05± 0.70	5.77± 1.17	6.92 ± 1.82	6.52 ±0.71	3.22± 1.66
Total Protein (mg\dL)	7.79± 0.44	10.34± 1.07	10.17± 1.23	8.93± 1.23	9.85± 1.57
Albumen (mg\dL)	2.82 ±0.52	3.21± 0.27	3.70± 0.36	3.45± 0.43	4.60± 0.45
Globulin (mg\dL)	4.96± 0.67	7.18± 1.18	6.77± 1.42	5.48± 1.14	5.25± 1.33
GPT (U/L)	11.59± 6.62	10.79± 5.14	11.52± 2.90	15.31± 5.28	69.78± 59.15
GOT (U/L)	3.93± 0.56	30.18± 8.87	28± 7.71	3.06± 1.15	15.53± 5.14

Table 2 : Effect of ciprofloxacin on Liver and Kidney function

Test	Control	Normal dose Short time	Over dose Short time	Normal dose Long time	Over dose Long time
ALKPase (U/L)	119.27± 19.87	198± 44.93	104.50± 17.74	64.35± 13.14	117.48± 31.24
ACPase (U/L)	5.35± 0.73	3.34± 0.99	10.62± 4.99	4.50± 1.53	4.95± 0.69
Total Protein (mg\dL)	8.16± 0.43	8.60± 1.14	11.55± 1.07	8.52± 0.65	8.34± 0.78
Albumen (mg\dL)	3.12± 0.70	2.79± 0.33	2.79± 0.25	2.80± 0.29	3.58± 0.49
Globulin (mg\dL)	5.04± 0.77	6.09± 1.24	8.75± 1.09	5.72± 0.76	4.75± 0.92
GPT (U/L)	5.03± 3.30	15.82± 5.54	7.21± 1.53	9.84 ±3.82	14± 8.80
GOT (U/L)	14.43± 2.80	34.56± 7.45	17.35± 3.45	26.90± 12.18	14.43± 3.65

Table 3 Effect of oxytetracycline on Liver and Kidney function

Test	Control	Normal dose Short time	Over dose Short time	Normal dose Long time	Over dose Long time
ALKPase (U/L)	81.55± 25.89	132± 26.24	114.29± 16.3	83.82± 22.85	50.49± 8.92
ACPase (U/L)	5.03± 0.61	5.35± 1.85	7.25± 1.17	18.52± 14.84	15.67± 9.18
Total Protein (mg\dL)	7.85± 0.26	9.56± 0.66	11.55± 1	7.48± 0.54	6.73± 0.60
Albumen (mg\dL)	2.40± 0.43	5.03± 0.62	4.22± 0.40	2.58±0.21	4.21± 0.48
Globulin (mg\dL)	5.44± 0.59	4.75± 0.66	6.89± 1.18	4.89± 0.50	2.52± 0.72
GPT (U/L)	18.02± 7.28	10.13± 2.10	17.28±9.19	47.68± 25.6	12.46± 5.24
GOT (U/L)	5.25± 1.14	23.77± 5.39	19.18± 5.0	8.75± 5.35	8.75± 4.47

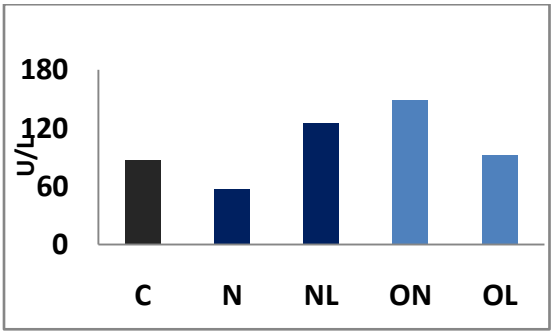

Figure 1A : ALKPase activity at different tylosine doses

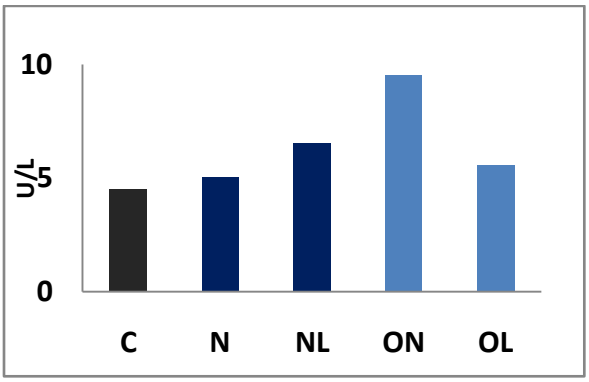

Figure 1B: ACPase Activity at different tylosine doses

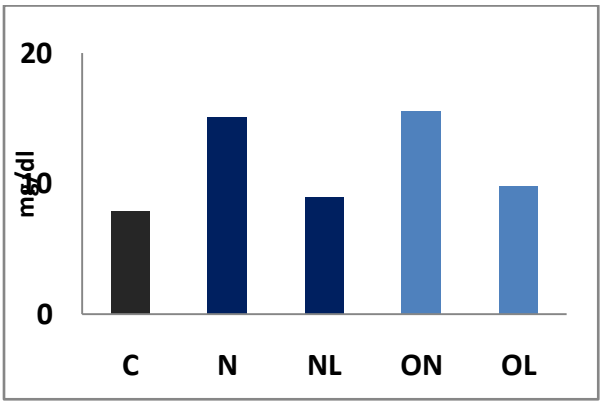

Figure 1C: Total protein under different tylosine doses

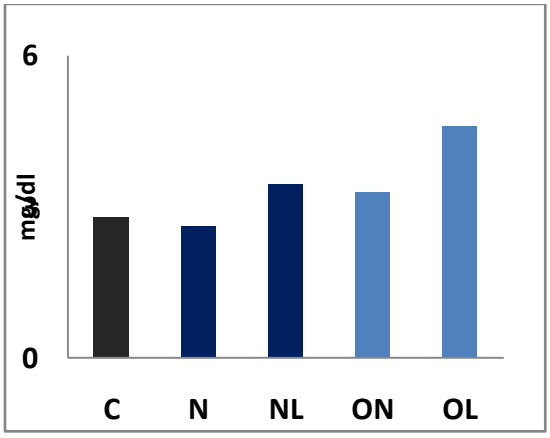

Figure 1D : Albumin concentration under different tylosine d doses

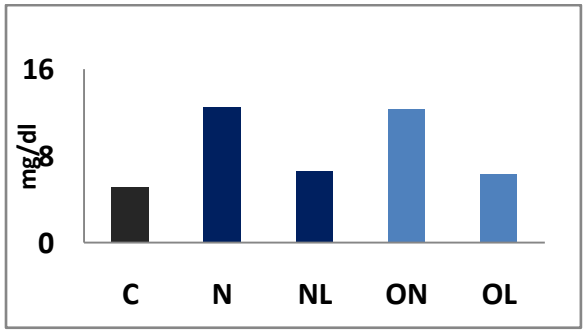

Figure 1E: Globulin concentration under different tylosine doses

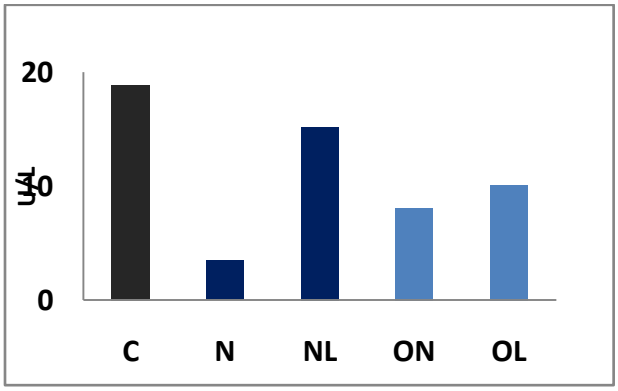

Figure 1F: ALT activity under different tylosine doses

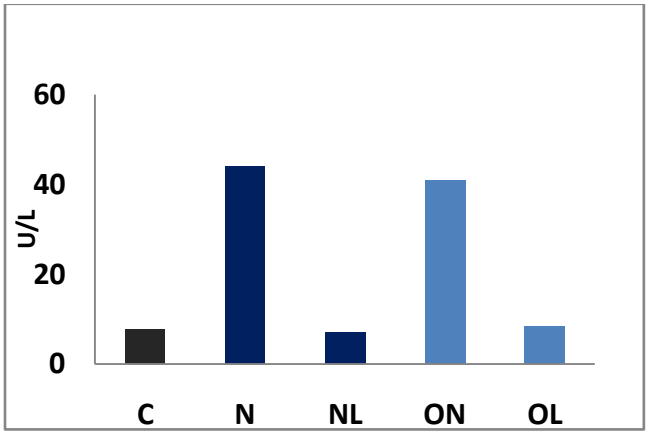

Figure 1G: AST activity under different tylosine doses

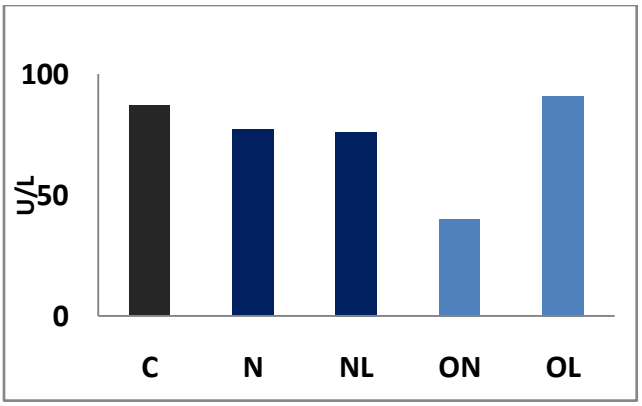

Figure 2A: AlkPase activity under different ciprofloxacin doses

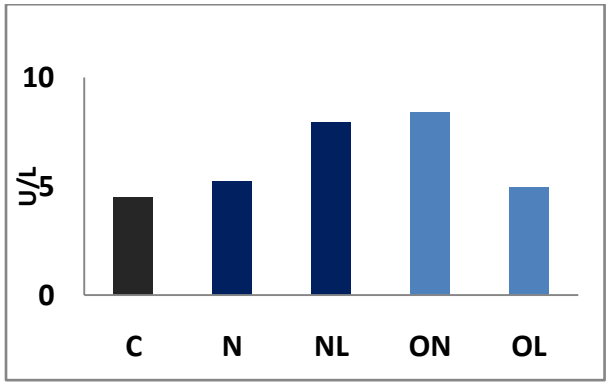

Figure 2B: ACPase activity under different ciprofloxacin doses

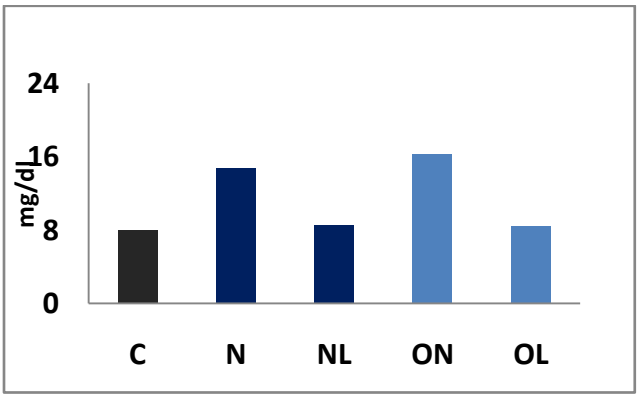

Figure 2C: Total protein activity under different ciprofloxacin doses

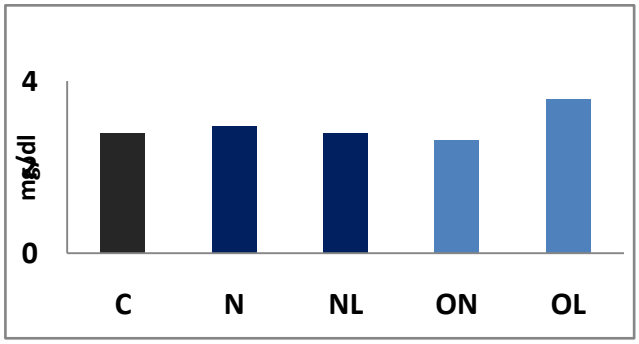

Figure 2D: Albumin activity under different ciprofloxacin doses

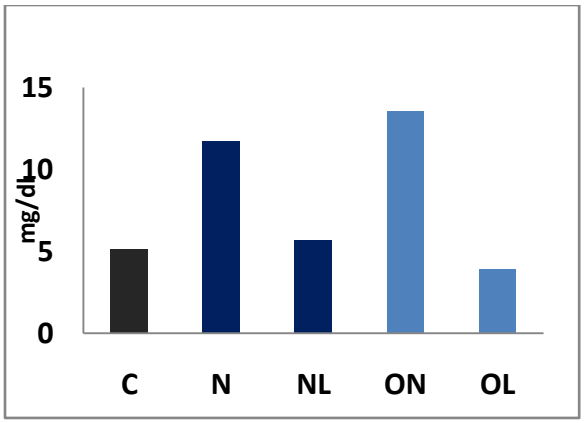

Figure 2E: Globulin activity under different ciprofloxacin doses

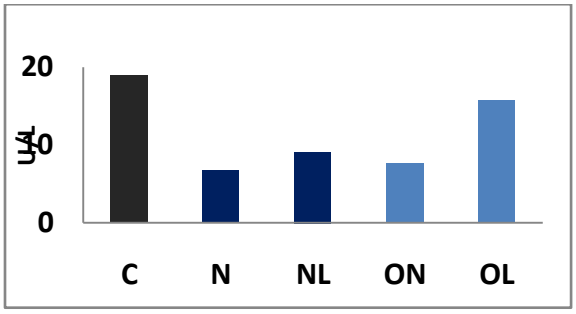

Figure 2F: ALT activity under different ciprofloxacin doses

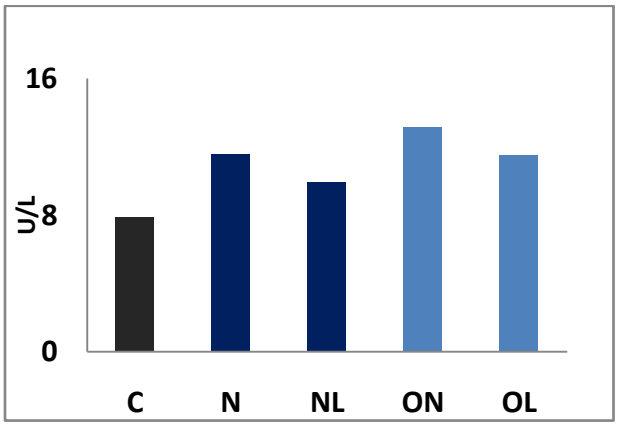

Figure 2G: AST activity under different ciprofloxacin doses

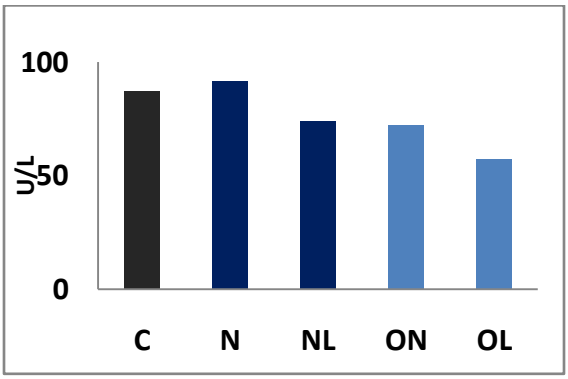

Figure 3A: AlkPase activity under different oxytetracycline doses

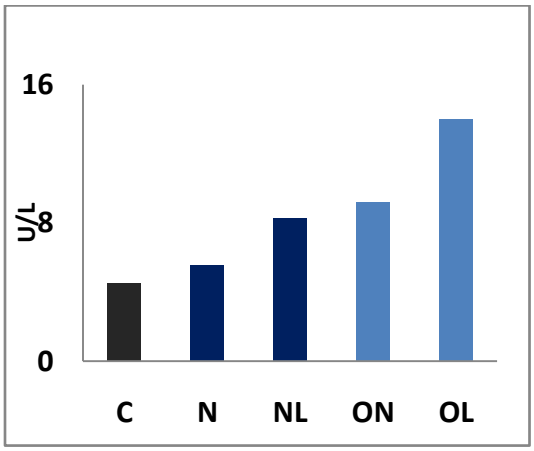

Figure 3B: ACPase activity under different oxytetracycline doses

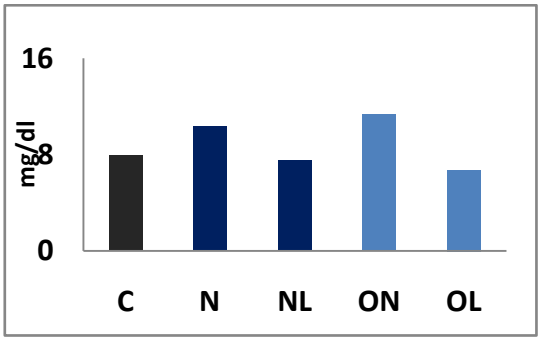

Figure 3C: Total protein activity under different oxytetracycline doses

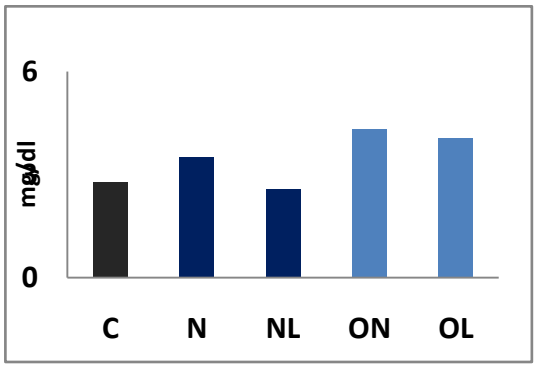

Figure 3D: Albumin activity under different oxytetracycline doses

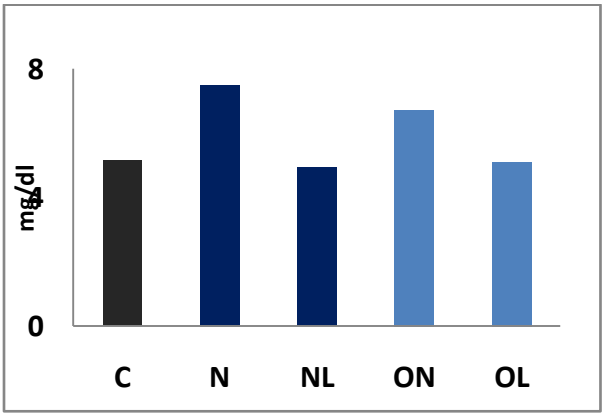

Figure 3E: Globulin activity under different oxytetracycline doses

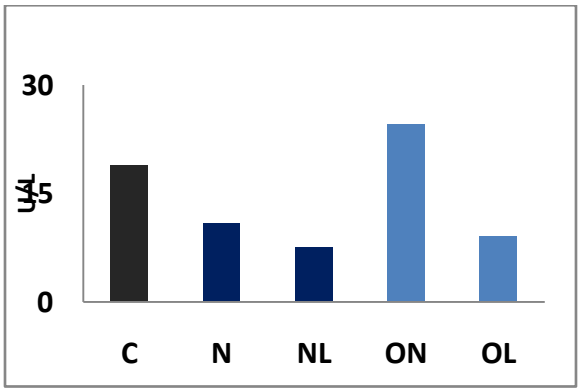

Figure 3F: ALT activity under different oxytetracycline doses

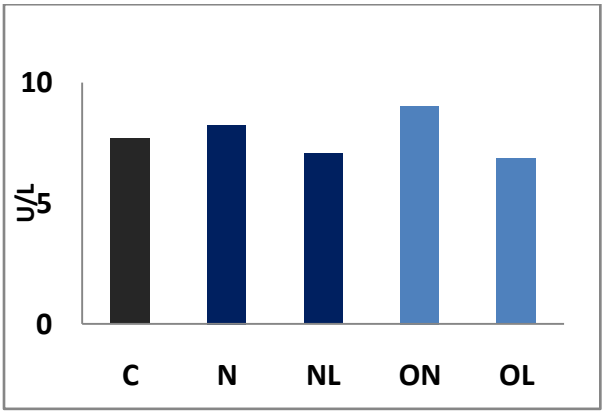

Figure 3G: AST activity under different oxytetracycline doses

Effect of tylosin on chicken liver and kidney functions

As it could be noticed in figures (1A-G) and Table 1 chicken received normal dose for normal duration exhibited increases almost in all tested proteins, however with a significant increase in ALKPase and total protein (p = 0.002) for roosters which received normal dose for long duration, and a decrease in case of ALT and AST. Chicken received over dose for normal duration showed a decrease only in case of ALT, and significant increases in ALKPase and total protein (p = 0.01, 0.004 respectively). Chicken treated with over dose for long duration showed increases in all tested proteins except a decrease in ALT.

Effect of ciprofloxacin on chicken liver and kidney functions

Chickens received normal dose of ciprofloxacin for normal duration exhibited, as compared to control, significant decreases in ALKPase (p = 0.003). On the other hand, in the same manner for normal dose for long duration, roosters which received normal dose for long duration reported no significant increases almost in all tested proteins. Roosters treated with over dose for normal duration showed also apparent decrease in ALKPase , ALT except albumin, in which the former remained slightly low. Roosters received over dose for long duration exhibited non significant increases almost in all tested biomarkers (figures 2A-G) and Table 2.

Effect of oxytetracycline on liver and kidney functions

Figures (3A-G) and Table 3 show the effect of dose and administration duration of oxytetracycline on chicken kidneys and liver biomarkers. Chickens which received normal dose of oxytetracycline for normal duration exhibited significant increases in ALKPase, total protein (p = 0.003 and 0.001 respectively), however with increase, though sometimes slight, in albumin. On the other hand, chickens received normal dose for long duration showed high significant decrease in ALKPase, (p = 0.003) and slight decrease in albumin (p = 0.020). A decrease, as compared to control, was also noted in case of total protein, globulin, ALT and AST.

Roosters which received over dose for normal duration exhibited significant increases in ACPase, total protein and albumin (p = 0.047, 0.027 and 0.014 respectively), however, with a decrease in ALKPase.

Roosters treated with over dose for prolonged duration showed major decrease in ALKPase and ALT activities. However, with a significant increase in ACPase activity (p = 0.021).

Hispathological effect of antibiotics on liver, kidney and heart

The adverse impact of antibiotics at varying dose and duration on chickens' liver, kidney, and heart is examined by histopathological studies and is exhibited by figures A-1, A-2 and A-3.

DISCUSSION

The misuse and self medication with antibiotics is a wide practice in Sudan, many of third as well as developed world countries [6]. However the problem is evident in low- and middle-income countries, where antibiotics are easily obtained as over the counter drug. Therefore, use of antibiotics without proper medical prescription is alarmingly high worldwide [5, 25].

In this study we have intentionally chosen three antibiotics that belong to varying classes of antibiotics. The experiments were designed and conducted in 78 roosters. Throughout the experimental tenure the birds showed normal activities with no sign of any illness. One of the antibiotics used in this study was tylosine which is primarily used in veterinary medicine; however it belongs to the same family of the humans widely used erythromycin [26]. We also used ciprofloxacin which belongs to fluoroquinolone drug class; it kills bacteria by stopping protein synthesis. Lastly we used oxytetracycline which works by interfering with the ability of bacteria to produce its essential proteins. For consistency purposes, only male chickens (roosters) with exactly similar ages were used in the study.

As far as frequency of doses and duration of different antibiotics administrated are concerned, results with different monitored biomarkers differed drastically. Obtained results are shown in the provided tables and figures above, which reflect the effect of antibiotics on liver biomarkers. Previous study on chickens liver biomarkers had shown obvious variations proteins such as AST (GOT), acid and alkaline phosphatase, ALT (GPT), total protein and globulin biomarkers. These variations had been attributed to variations in sex, age, environmental conditions and dietary factors [enzyme levels in birds]. However, in our study since we have unified all of the above mentioned conditions, our results would emphasize directly on only antibiotics administration.

In this study, administration of all the three antibiotics in normal dose for normal duration, exhibited a clear increase in total protein, globulin and AST, however, a sharp decrease of up to

4 folds as compared to control was noticed with ALT. Interestingly, when these antibiotics were administered by roosters for normal dose, but, for long duration, an evident increase was noticed in the ALKPase activity in the tylosin administered antibiotic. Such elevation was not noticed when the antibiotic was administered at normal dose for normal duration. Since elevation of ALKPase activity is normally linked to liver injury [7], these results could therefore, hint for a possible direct adverse effect of this antibiotic when administered, though at normal dose for prolonged duration on liver functions.

Administration of antibiotics at over dose for normal duration resulted in fluctuated activities for the liver important biomarker ALKPase. These variations were either very below or above the control experiment. Such decrease or increase in this enzyme activity was not common in all antibiotics used in this experiment. Since the decrease or increase in ALKPase are commonly justifiable by a possible liver injury. These findings, may therefore, be linked to varying degrees of effect of antibiotics on liver and kidney functions.

Interestingly we have observed sudden and sharp increase or decrease in many of the liver and kidney proteins investigated in this study upon administration of antibiotics in normal dose for normal duration, a phenomenon not noticed even when the drug was administered at over dose for long duration. This could logically be attributed to the sudden first reaction of tissues to the administered drug.

Since the main focus of this study was on possible effect of misuse of antibiotic on liver and kidney functions, it would be more interesting to compare the effect of the administered antibiotics under misuse conditions of the drug; therefore this study was conducted in which roosters were treated with antibiotics under varying conditions such as 1) roosters received normal dose of antibiotics for normal duration 2) roosters received normal dose of antibiotic for prolonged period 3) roosters received overdose of antibiotic for normal period and 4) roosters received overdose of antibiotic for prolonged period.

Following the antibiotic administration for the prescribed duration and measurement of liver and kidney biomarkers such as ALKPase, ACPase, total protein, globulin, albumin, AST and ALT, the following interesting observations were noticed and will be discussed hereafter.

Chickens treated with ciprofloxacin

ALKPase activity showed significant decreased activity under normal dose for prolonged duration. The gradual decrease in this enzyme activity from therapeutic dose to normal dose for prolonged duration to over dose for normal duration may indicate the effect of both dose as well as the duration of administration (Figure A-1)

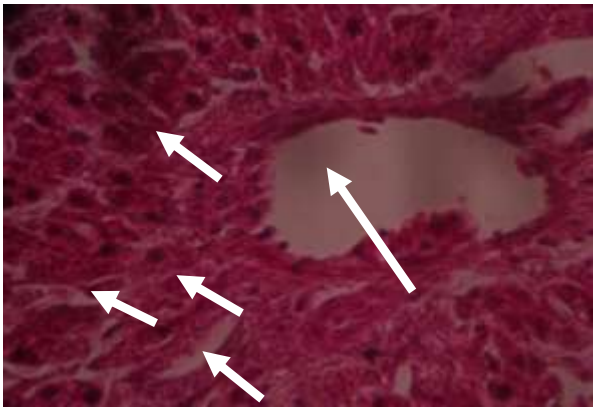

Figure A-1: Effect of ciprofloxacin on hepatic tissues of roosters received normal dose of ciprofloxacin for long duration. Short Arrows indicate positions of hepatocellular degeneration and centrilobular hepatocellular. While long arrow indicates pressure atrophy and dilated congested central vein.

Administration of chloramphenicol to chicken under therapeutic dose had shown to affect ALKPase, however, after 4 weeks of this administered dose, activity of enzyme was restored [27]. ALKPase had also been used as a sign for liver damage when experimental rats were treated with antitubercular drugs such as rifampicin [28]. ACPase exhibited significant decrease or increase in activity as compared to control under varying experimental conditions at varying antibiotics used. Our results with ACPase activity could justify the rare literatures that link this enzyme with liver or kidney damage. On the other hand, ALT and AST activities were clearly higher than control under almost all experimental doses and durations. These two enzymes had been repeatedly associated with malfunction of kidney and liver[29-31]. Previous literatures had linked a decreased in ALT to associate with liver cirrhosis [32]. In an experiment conducted in different genders of mice, *Bocker et al* had reported a pronounced increase in AST and ALT of female mice treated with tetracycline over males mice [33].

Chickens treated with oxytetracycline

As compared to control, ALKPase, ACPase, ALT and AST exhibited clear either decrease or increase in activity as compared to control. Our results, partially, are in accordance with Shaddad and his co-workers who worked on a similar type of investigation on administration of different doses of oxtetracycline on poultry growth and metabolism. They had reported an increase in the level of both AST and ALT. The same authors observed liver pathological conditions upon administration of this antibiotic at high dose [34]. In this study, the histopathological studies, indicated clear centrilobular hepatocellular bleeding which could account for the obtained disturbances in ALKPase, ACPase, ALT and AST (figure A-2)

Figure A-2: Effect of tylosine on hepatic cells of roosters received over dose of oxytetracycline for normal and long duration. Arrows indicate positions of hepatocellular bleeding

Saraswat and his colleagues demonstrated sever liver damage when oxytetracycline was administered at high dose of 200mg/kg to experimental rats [35].

Chickens treated with tylosin

Though tylosin is a veterinarian antibiotic and is not directly administered by human, it still represents one of the direct hazardous antibiotic to human health through human ingested animal tissues, chicken meat, eggs or even milk [36-39]. On the other hand, since this antibiotic belongs to the same family of the human widely consumed erythromycin, the obtained results with tylosin could still be extrapolated to human. In the current investigation, consistent increase or decrease was apparent in ALKPase, ALT and AST. Clear decrease was seen in the total protein, ALT activities in the roosters which received overdose antibiotic for long duration, which may indicate a chronic effect of both dose and duration on liver functions (figure A-3).

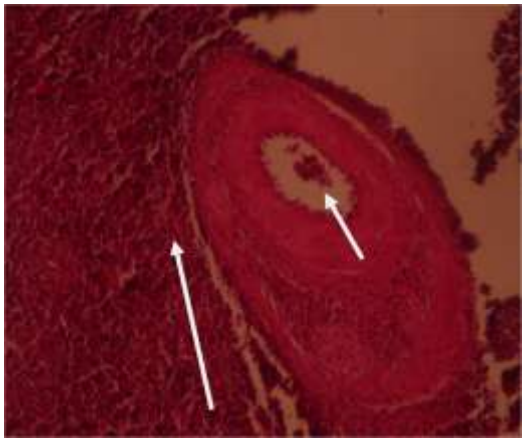

Figure A-3: Effect of tylosine on hepatic cells of roosters received normal dose of tylosine for long duration. Short Arrow indicates congestion of portal vein tributaries. While long arrow shows portal tract triaditis.

Effect of dose and duration of administered antibiotic on liver functions

The liver has very complicated functions among which is the detoxification of drugs such as antibiotics and its metabolites. Some antibiotics can cause allergic reactions while others can cause direct damage to the liver; such liver injury is usually difficultly diagnosed due to absence of a specific biomarker [40]. In addition, if the kidneys are already weak, some antibiotics can harm them further [41]. Based on these previous reports we have planned our experiments to study effect of both dose and duration of administered drug on liver and kidney functions. Though our results was little difficult, in some cases, to analyze due to enzymes activity variations, in some cases, clear effects for over dose as well as prolonged duration of administration were evident. For instance, when two groups of roosters were treated with tylosin for normal duration, however one group was treated with normal dose while the other group was treated with over dose. ALKPase was evidently one fold higher in case of group received over dose than the one treated with normal dose. Similar results were not obtained when this enzyme was followed in those roosters which received similar dose of oxytetracycline and ciprofloxacin. ALKPase activity was higher in the group treated with over dose ciprofloxacin for prolonged duration over the group treated with normal dose however for prolonged duration. In addition to the evidenced effects of the over dose on liver ALKPase, when a normal dose of tylosin was administered for little longer duration, an increase in the ALT was clear as compared to normal therapeutic dose. Similar results, with the same enzyme, were noticed with ciprofloxacin, however with also, in addition to effect of the dose, a clear effect of the duration of treatment.

In order to study the elimination of the administered antibiotic residual plasma concentration was followed. Based on microbiological data, we detected no residues tylosin in chicken plasma after withdrawal period (defined as time duration which passes between the last dose given to the animal and the time when the level of residue in the tissue is lower than or equal to maximum residue limits), the concentrations of tylosin normal dose and over dose in plasma were 0.56µg/ml , 0.57µg/ml, this result is within the allowed maximum limit of 1.2 µg/ml [42].

Effect of antibiotics on heart

Roosters treated with over dose of ciprofloxacin for long duration exhibited cardiac bleeding and degeneration Figure 4-A.

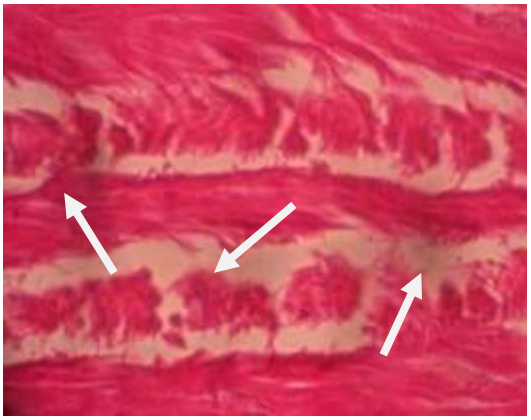

Figure 4-A: effect of ciprofloxacin on heart tissues of roosters received over dose of ciprofloxacin for long duration. Arrows show myocardial degeneration and bleeding intermuscular spaces and hyperaemia.

Prabhakar and Krahn reported an unexplained sudden temporarily cardiac arrest upon administration of ciprofloxacin [43], this arrest was thought to be due to the effect of ciprofloxacin in prolonging QT interval. Considering the histopathological data which here depicted and Prabhakar and Krah results , it could be concluded that administration of ciprofloxacin could be fatal especially for patients who use medications meant for prolonging QT wave intervals [44].

CONCLUSIONS

From this study we could conclude that regardless of antibiotic group or type, all antibiotics when administered either at high dose or normal dose but for long duration cause an apparent change in the liver and kidneys physiological and metabolic activities, and could therefore be a logical reason behind these vital organs diseases or malfunctioning.

REFERENCE

1. Schwalbe R, Steele-Moore L, Goodwin AC, editors. Antimicrobial Susceptibility Testing Protocols: CRC Press; 2007
2. Finberg RW, Moellering RC, Tally FP, Craig WA, Pankey GA, Dellinger EP, et al. The importance of bactericidal drugs: future directions in infectious disease. Clin Infect Dis. 2004; 39(9): 1314-20.
3. Reig M, Toldra F. Veterinary drug residues in meat: Concerns and rapid methods for detection. Meat Sci. 2008; 78(1-2): 60-7.
4. Walsh C. Antibiotics: Actions, Origins, Resistance: ASM Press 2003
5. Awad A, Eltayeb I, Matowe L, Thalib L. Self-medication with antibiotics and antimalarials in the community of Khartoum State, Sudan. J Pharm Pharm Sci. 2005; 8(2): 326-31.
6. Awad AI, Eltayeb IB. Self-medication practices with antibiotics and antimalarials among

Sudanese undergraduate university students. Ann Pharmacother. 2007; 41(7): 1249-55.

7. Dhawale A. The Liver: a big organ with a big role. World poultry. Nagpur; 2007.
8. Berk PD, KM K. Approach to the patient with jaundice or abnormal liver test results. In: Goldman L, Schafer AI, editors. Philadelphia: Saunders Elsevier; 2011.
9. Pratt DS. Liver chemistry and function tests. In: Feldman M FL, Brandt LJ, eds., editor. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. Philadelphia, PA: Saunders Elsevier; 2010.
10. Wilcox FH. Phosphatases in chicken semen. J Reprod Fertil. 1961; 2: 148-51.
11. Griminger P, Scanes CG. Protein Metabolism. In: Sturkie PD, editor. Avian Physiology: Springer New York; 1986. p. 326-44.
12. Murray R .Alanine aminotransferase. In: Kaplan A and Peace AL, editor. Clinical Chemistry St Louis, Toronto, Princeton: The C. V. Mosby Co; 1984. p. 1088-90.
13. Garcia Ovando H, Gorla N, Luders C, Poloni G, Errecalde C, Prieto G, et al. Comparative pharmacokinetics of enrofloxacin and ciprofloxacin in chickens. J Vet Pharmacol Ther. 1999; 22(3): 209-12.
14. el-Gendi AY, el-Banna HA, Abo Norag M, Gaber M. Disposition kinetics of danofloxacin and ciprofloxacin in broiler chickens. Dtsch Tierarztl Wochenschr. 2001; 1.34-429 :(10)08
15. Arzey GG, Arzey KE. Successful treatment of mycoplasmosis in layer chickens with single dose therapy. Aust Vet J. 1992; 69(6): 126-8.
16. Singh M, Varshneya C, Telang RS, Srivastava AK. Alteration of pharmacokinetics of oxytetracycline following oral administration of Piper longum in hens. J Vet Sci. 2005; 6(3): 197-200.
17. Bruce AW, Mahan DE, Morales A, Clark AF, Belville WE. An objective look at acid phosphatase determinations: a comparison of biochemical and immunological methods .Br J Urol. 1979; 51(3): 213-7.
18. Sommer AJ. The determination of acid and alkaline phosphatase using p-nitrophenyl phosphate as substrate. Am J Med Technol. 1954; 20(4): 244-53.
19. Petera V, Bobek K, Lahn V. Serum transaminase (GOT, GPT) and lactic dehydrogenase activity during treatment with methyl testosterone. Clin Chim Acta. 1962; 7: 604-6.
20. Henry RJ, Sobel C, Berkman S. Interferences with Biuret Methods for Serum Proteins Use of Benedict's Qualitative Glucose Reagent as a Biuret Reagent. Analytical Chemistry. 1957; 29(10): 1491-5.
21. Kessler MA, Meinitzer A, Wolfbeis OS. Albumin Blue580 Fluorescence Assay for Albumin.

Analytical Biochemistry. 1997; 248(1): 180-2.

22. Van-Leeuwen F. Tylosin. In: Toxicological Evaluation of Certain Veterinary Drug Residues in Food. Geneva; 1991
23. Luna LG, editor. Manual of Histologic Staining Methods ; of the Armed Forces Institute of Pathology. 3 ed: Blakiston Division, McGraw-Hil; 1968.
24. Taksir TV, Johnson J, Maloney CL, Yandl E, Griffiths D, Thurberg BL, et al. Optimization of a histopathological biomarker for sphingomyelin accumulation in acid sphingomyelinase deficiency. J Histochem Cytochem. 2012; 60(8): 620-9.
25. Mainous AG, 3rd, Cheng AY, Garr RC, Tilley BC, Everett CJ, McKee MD. Nonprescribed antimicrobial drugs in Latino community, South Carolina. Emerg Infect Dis. 2005; 11(6): 883-8.
26. Anadon A, Reeve-johnson L. Macrolide antibiotics, drug interactions and microsomal enzymes: implications for veterinary medicine. Res Vet Sci. 1999; 66(3): 1.203-97
27. Cybulska R, Jaworska-Adamu J, Cybulski W. Effect of chloramphenicol on the activity of alkaline phosphatase (EC 3.1.3.1.) and the structure of microvilli of the epithelial cells of the small intestine of the hen. Folia Histochem Cytobiol. 1988.61-257 :(4)26 ;
28. Pari L, Kumar NA. Hepatoprotective activity of Moringa oleifera on antitubercular drug-induced liver damage in rats. J Med Food. 2002; 5(3): 171-7.
29. Sharma S, Goyal RP, Chakravarty G, Sharma A. Toxicity of tomato red, a popular food dye blend on male albino mice. Experimental and Toxicologic Pathology. 2008; 60(1): 51-7.
30. Frejo MT, Del Pino J, Lobo M, Garcia J, Capo MA, Diaz MJ. Liver and kidney damage induced by 4-aminopyridine in a repeated dose (28 days) oral toxicity study in rats: gene expression profile of hybrid cell death. Toxicol Lett. 2014; 225(2): 252-63.
31. Palipoch S, Punsawad C. Biochemical and histological study of rat liver and kidney injury induced by Cisplatin. J Toxicol Pathol. 2013; 26(3): 293-9.
32. Takahashi A, Sekiya C, Yazaki Y, Ono M, Sato H, Hasebe C, et al. Hepatic GOT and GPT activities in patients with various liver diseases--especially alcoholic liver disease. [Hokkaido igaku zasshi] The Hokkaido journal of medical science. 1986; 61(3): 431-6.
33. Böcker R, Estler CJ. A high pressure liquid chromatographic method for the determination of tetracyclines in blood and organs of experimental animals. Arzneimittel-Forschung. 1979; 29(11): 1690-3.
34. Shaddad SA, Wasfi IA, Maglad MA, Adam SE. The effect of oxytetracycline on growth and lipid metabolism in poultry. Comp Biochem Physiol C. 1985; 80(2): 375-80.

35. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Protective effect of picroliv, active constituent of Picrorhiza kurrooa, against oxytetracycline induced hepatic damage. Indian J Exp Biol. 1997; 35(12): 1302-5.
36. Lopes RP, Reyes RC, Romero-Gonzalez R, Frenich AG, Vidal JL. Development and validation of a multiclass method for the determination of veterinary drug residues in chicken by ultra high performance liquid chromatography-tandem mass spectrometry. Talanta. 2012; 89: 201-8.
37. Hamscher G, Limsuwan S, Tansakul N, Kietzmann M. Quantitative analysis of tylosin in eggs by high performance liquid chromatography with electrospray ionization tandem mass spectrometry: residue depletion kinetics after administration via feed and drinking water in laying hens. J Agric Food Chem. 2006; 54(24): 9017-23.
38. Roudaut B, Moretain JP. Residues of macrolide antibiotics in eggs following medication of laying hens. Br Poult Sci. 1990; 31(3): 661-75.
39. Peng D, Ye S, Wang Y, Chen D, Tao Y, Huang L, et al. Development and validation of an indirect competitive enzyme-linked immunosorbent assay for the screening of tylosin and tilmicosin in muscle, liver, milk, honey and eggs. J Agric Food Chem. 2012; 60(1): 44-51.
40. Abenavoli L, Libri E, Bosco D, Gallo D, Luzza F. [Drug-induced liver injury]. Recenti Prog Med. 2012; 103(2): 79-84.
41. Khalili H, Bairami S, Kargar M. Antibiotics induced acute kidney injury: incidence, risk factors, onset time and outcome. Acta Med Iran. 2013; 51(12): 871-8.
42. Kowalski C, Rolinski Z, Zan R, Wawron W. Pharmacokinetics of tylosin in broiler chickens. Pol J Vet Sci. 2002; 5(3): 127-30.
43. Prabhakar M, Krahn AD. Ciprofloxacin-induced acquired long QT syndrome. Heart Rhythm. 2004; 1(5): 624-6.
44. Ibrahim M, Omar B. Ciprofloxacin-induced torsade de pointes. The American journal of emergency medicine. 2012; 30(1): 252.e5-.e9.