Comparative safety profiles of two enrofloxacin generics after repeated intracrop administration to broilers

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Abstract
Clinicochemical, haematological and histopathological alterations were demonstrated in broiler chickens following repeated oral bolus administration of two different enrofloxacin generic preparations, formulated as 10% oral solutions, given at a dose regimen of 10 mg/Kg body weight for 5 consecutive days. The two tested preparations were EnroFloc® (Medmac®, Jordan) referred thereafter as ENRO-A; and Syvaquinol® (Syva®, Spain) which referred thereafter as ENRO-B. Eighteen broilers chickens, aging 40 days old, divided equally and randomly into three groups, have been used in the present study. ENRO-A or ENRO-B was given intracrop route of administration at the above-mentioned dose regimen to birds of the 2nd and 3rd groups, respectively; whereas those of the 1st group were given water instead and kept as control. Blood samples were collected from all birds via the wing and metatarsal veins on the 5th day for clinicochemical and haematological examinations. Birds were then humanely sacrificed and liver, kidneys and heart were dissected out for histopathological examination. Results revealed that ENRO-A induced a significant (p<0.05) increase of the activity of alkaline phosphatase compared to ENRO-B as well as control group. Both ENRO-A and ENRO-B caused significant increases in the levels of plasma urea and creatinine concentrations compared to control (p<0.05), with higher significance in case of ENRO-A. Activity of plasma creatine kinase significantly (p<0.05) increased after ENRO-A compared to control and ENRO-B-treated groups. ENRO-A and ENRO-B significantly (p<0.05) increased blood glucose and triglyceride levels compared to that of control. Cholesterol level was increased significantly (p<0.05) only after ENRO-B repeated administration. However, other metabolic parameters showed insignificant changes. Parallel inflammatory and degenerative histopathological changes in the affected organs, except kidneys, have been observed. Nevertheless, administration of either ENRO-A or ENRO-B caused insignificant changes in hematological parameters of the treated chicken groups. Data of the present study may indicate that enrofloxacin may cause organ dysfunction in broilers during the course of therapy based on clinicochemical and histopathological reasons. The data may also indicate that the pharmaceutical technology may be a detrimental factor in safety profiles of generic products based on the differences recorded between the two tested brands.

Keywords: Broiler Chicken; Enrofloxacin; Pharmacovigilance; Safety.

1. Introduction
Generic drug preparations became abundant nowadays from different sources with different efficacies, safety profiles and prices. However, not all preparations give the same desired effects and safety levels in the veterinary medical field. The term ‘generic product’ used by the World Health Organization (WHO) for a pharmaceutical product that is: i) intended to be interchangeable with the innovator product in an individual patient, ii) usually manufactured without a licence from the innovator company, and iii) marketed after expiry of patent or other exclusivity rights (Birkett 2003). Comparative clinical pharmacology studies are conducted by pharmacologists for the comparison of different medicinal products containing the same active substance. Enrofloxacin (ENRO) is a synthetic chemotherapeutic agent, belonging to the second generation of the fluoroquinolone class, a derivative of quinolonecarboxylic acid group, used as antibacterial to combat infections. ENRO has a broad spectrum bactericidal activity against Gram-positive and Gram-negative bacteria (Merk Index 2001). ENRO was developed exclusively for veterinary use in many animal species including, cattle, pigs, dogs, cats… etc. (Altruithr 1987); and for the treatment of respiratory disease in chickens and turkeys (Anderson et al. 2003). After administration, ENRO is partly de-ethylated to ciprofloxacin in vivo, which is also pharmacologically active and is employed in human medicine (Rao et al. 2002). Although FDA withdrew the approval for ENRO for the purpose of treating bacterial infections in poultry because of scientific data that showed that the use of ENRO in poultry caused resistance to Campylobacter, a bacterium that causes foodborne illness (FDA 2005). However, in many developing countries, ENRO is being used as the routine choice to treat almost any bacterial disease in poultry. Like all members of fluoroquinolones, ENRO produces its antimicrobial action in multiple species of bacteria via damage of bacterial DNA leading to defects in negative supercoiling. This effect was linked to inhibition of DNA gyrase activity, an enzyme found in all bacteria (Gellert et al. 1977).
The original preparation of ENRO was produced by Bayer® Corporation, Animal Health Division (Barmen, Germany) under the trade name of Baytril® of different pharmaceutical formulations. However, nowadays, there are many generic preparations of ENRO marketed in the veterinary medical drug markets and not all preparations give the same desired effects and safety levels. As the potential non-equivalence of a drug preparation has been a matter of pharmacological concern, therefore, the aim of the present study was to compare between the safety profiles of two ENRO preparations (ENRO-A and ENRO-B) that are widely distributed in the veterinary drug market for broiler industry after repeated intracrop (i.c.) administration; and giving recommendations to the concerned veterinary authorities, veterinarians and farmers about the elected brand from the pharmacovigilance point of view.

2. Materials and methods

2.1. Drugs

ENRO occurs as a pale yellow crystalline powder slightly soluble in water at pH = 7, soluble in potassium hydroxide, glacial acetic acid, ethanol, 0.1 M hydrochloric acid, 0.1 M sodium hydroxide (Heitzman 1997). ENRO has the following structural formula (Figure 1) and specifications:

![Structural Formula of Enrofloxacin](image)

ENRO has the molecular formula C_{18}H_{19}FN_{5}O_{5}, the chemical Name: 1-cyclopropyl-7-(4-ethyl-1-piprazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinoine carboxylic acid; and molecular weight: 359.39 g/mole. Two generic ENRO oral preparations were used in the present study. They were obtained from our local drug market as 10% solutions. Brand-A was Enrofloxacin® 10 oral solution, which contains ENRO at concentration of 100 mg/ml; the preparation is a product of Medmac® (Leon, Spain); Batch: v210804. While Brand-B was Syvaquin® 10% oral solution, which also contains ENRO at concentration of 100 mg/ml; the preparation is a product of Syva® (Leon, Spain); Batch: 301216.

2.2. Experimental animals

Eighteen clinically healthy Ross broiler chickens were used. Animals were numbered and accommodated in a suitable pen under hygienic condition with controlled temperature (22±1°C), humidity (60±10%) and light (12 h per day) for at least a week before being used. Food and water were available ad libitum. Feed was withdrawn 12 hours before drug administration to avoid absorption variability due to possible ENRO-feed interaction. Water was withdrawn 2 hour to minimize variation in stomach emptying or degree of ENRO. The chickens were weighed prior to experiment for dose adjustment; the average body weight was 2.5 Kg.

The experiment was designed in a parallel manner where the chickens were grouped randomly into three groups; Group (I): included 6 chickens; each bird was orally (i.c.) administered repeated doses of distilled water for 5 consecutive days; and kept as control; Group (II): included 6 chickens; each bird was orally (i.c.) administered repeated doses of 10 mg/kg body weight of ENRO 10% (Brand-A) for 5 consecutive days; and Group (III): included 6 chickens; each bird was orally (i.c.) administered repeated doses of 10 mg/kg body weight of ENRO 10% (Brand-B) for 5 consecutive days.

2.3. Drug preparation and administration

The recommended dose of ENRO in poultry is 10 mg/kg body weight by various routes of administration. Each of the two commercial oral preparations was already formulated as ENRO 10% (100 mg/ml). Experimentally, an oral solution of ENRO 1% (10 mg/ml) of both preparations (A) and (B) was prepared by adding 1.0 ml of the corresponding stock solution of ENRO 10% to 9.0 ml of bi-distilled water. Administration via oral route was performed using a syringe with plastic cannula to deliver the drug solution i.e.

2.4. Sampling

On the fifth day, two hours post last dose, three ml of blood were drawn from each bird in into heparinized tubes properly labelled with group and chicken numbers. Each sample was divided into 2 parts; the first part was kept a whole blood that was used for hematology; while the second part was centrifuged at 3000 rpm for 10 minutes to separate plasma for biochemical investigations.

After blood collection, the chickens of each group were ethically sacrificed and specimens from liver, kidneys and heart were picked out for histopathological examination.

2.5. Assays

2.5.1. Haematological assay

To assess the blood safety profile of examined brands of ENRO (A & B) in broilers, the following haematological parameters in control and treated (10 mg/kg daily for 5 consecutive days, i.c.) chicken samples were automatically evaluated by auto-hematology analyser (Mindray®, Model BC-2800Vet, Shenzhen, China). Erythrocytic parameters included red blood cell (RBC) count, haematocrit value (HCT), mean corpuscular volume (MCV), haemoglobin concentration (HGB), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Leukocytic parameters included white blood cell (WBC) count, differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), platelets’ (PLT) concentration, platelet distribution width (PDW), and mean platelet volume (MPV). Usually in blood specimens, the cells are too close to each other to be identified or measured. For this reason, the special provided diluent was used to separate the cells so that they are drawn through the aperture of the auto-analysier once a time as a well as to create a conductive environment for blood analysis. When analysing a whole blood sample, the analyser aspirates 13 μL of the sample and dilutes it (1: 308 for WBCs and 1: 44862 for RBCs) before proceeding to the actual analysis. After reacting with the diluent and lyse, the cells mainly fall into the following three volume ranges: WBC, 30~350 fl; RBC: 25~250 fl; PLT: 2~30 fl. With the help of the diluent and lysis buffer, the analyser can size the white cells into three sub-populations, lymphocytes, mid-sized cells, and granulocytes. The analyser adopts the Coulter Principle to count RBC, WBC, and PLT cells and to draw their corresponding histograms. The HGB concentration is obtained by the colorimetric method while the MCV and MPV are calculated electronically. The rest of indices are mathematically derived from those according to the following equations:

\[\text{HCT} \% = \frac{\text{RBC} (10^{12}/L) \times \text{MCV} (\text{fl})}{10}\]

\[\text{MCH} (pg) = \frac{\text{HGB} (g/dL) \times 10}{\text{RBC} (10^{12}/L)}\]

\[\text{MCHC} (g/dL) = \frac{\text{HGB} (g/dL) \times 100}{\text{HCT} \%}\]

Based on the WBC histogram, this analyser calculates Lymph%, Mid% and Gran% as follows:
Lymph (%) = \frac{PL}{PL + PM + PG} \times 100

Mid (%) = \frac{PM}{PL + PM + PG} \times 100

Gran (%) = \frac{PG}{PL + PM + PG} \times 100

Where, PL = particles in the lymphocyte region (10^9/L); PM = particles in the mid-size region (10^9/L); PG = particles in the granulocyte region (10^9/L).

Lymph# (10^9/L) = \frac{Lymph (%) \times WBC (10^9/L)}{100}

Mid# (10^9/L) = \frac{Mid (%) \times WBC (10^9/L)}{100}

Gran# (10^9/L) = \frac{Gran (%) \times WBC (10^9/L)}{100}

PLT count (10^9/L) is measured directly by counting the platelets passing through the aperture; and based on the PLT histogram; the analyser electronically calculates the mean platelet volume (MPV, fL) and platelet distribution width (PDW). PCT is derived from the following equation:

PCT (%) = \frac{PLT (10^9/L) \times MPV (fL)}{10}

2.5.2. Clinico-chemical assays

Estimating plasma clinicochemical parameters, evaluating hepatotoxicity, nephrotoxicity, cardiotoxicity and/or metabolism was carried out spectrophotometrically (Jenway®, Model 6500, Germany) using diagnostic kits purchased from Analyticon® Biotechnologies AG (Lichtenfels, Germany). The parameters were alkaline phosphatase (Bessey et al. 1946), aspartate aminotransferase (Bergmeyer et al. 1986), alanine aminotransferase (Bergmeyer et al. 1986), total protein (Tietz 1995), creatine kinase (Black et al. 1986), albumin (Marshall 1988), urea (Krieg et al. 1986), creatinine (Bartels et al. 1972), glucose (Schmidt 1961), and total cholesterol (Allain et al. 1974). Plasma triacylglycerols level was estimated using a diagnostic kit purchased from Biorbax® SA (Maizy, France) according to Fossati and Prencipe (1982). Globulin level was calculated by subtracting the value of albumin from the value of total protein according to Doumas and Biggs (1972).

2.5.3. Histopathological assay

The liver, heart and the two kidneys were taken from sacrificed birds in all groups and preserved in neutral formalin solution 10% for 24 hours of fixation, samples were dehydrated by using graded increased concentrations of ethyl alcohol and then embedded in melted paraffin wax that was set at P<0.05. After 24 hours of fixation, samples were washed by running water over night. The washed samples were embedded using a diagnostic kit purchased from Biorbax® SA (Maizy, France) according to Fossati and Prencipe (1982). Globulin level was calculated by subtracting the value of albumin from the value of total protein according to Doumas and Biggs (1972).

2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean of 6 observations (n). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA) followed by LSD as post-hoc test. P-values of 0.05 or less were considered significant. All statistical analytical procedures were done using SPSS software v.20 (Chicago, Illinois).

3. Results

Haematological, clinicochemical, and histopathological alterations were demonstrated in broiler chickens following repeated oral bolus administration of ENRO-A or ENRO-B at a dose rate of 10 mg/Kg body weight for 5 consecutive days. All data are expressed as Mean±SEM of 6 chickens and differences between ENRO-treated groups and corresponding control were determined using ANOVA followed by LSD as a post-hoc test; significance level was set at P<0.05.

3.1. Haemogram

Oral administration of either ENRO-A or ENRO-B caused insignificant changes in hematological parameters as presented in tables (1 & 2).

3.2. Clinico-chemical findings

Table 3 shows the effect of ENRO-A and ENRO-B (10 mg/Kg; intracrop; for 5 consecutive days) on organ (liver, kidney & heart) function profiles of broiler chickens compared to control (received only saline). Repeated oral administration of ENRO-A induced a significant (P<0.05) increase of the activity of alkaline phosphatase compared to ENRO-B as well as control group. Both ENRO-A and ENRO-B caused significant increase in levels of plasma urea and creatinine concentrations compared to control (P<0.05), with higher significance in case of ENRO-A. Activity of plasma creatine kinase significantly (P<0.05) increased after ENRO-A compared to control group and ENRO-B-treated group as well.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ENRO-A</th>
<th>ENRO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (10^9/L)</td>
<td>20.340 ± 1.90</td>
<td>20.298 ± 2.97</td>
<td>20.14 ± 2.22</td>
</tr>
<tr>
<td>Granulocytes (x10^9/L)</td>
<td>12.502 ± 0.970</td>
<td>12.198 ± 1.43</td>
<td>12.408 ± 1.65</td>
</tr>
<tr>
<td>Monocytes (x10^9/L)</td>
<td>3.530 ± 0.700</td>
<td>3.450 ± 1.250</td>
<td>3.35 ± 0.890</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>4.265 ± 0.949</td>
<td>4.660 ± 0.712</td>
<td>4.38 ± 0.651</td>
</tr>
<tr>
<td>PLT (x10^9/L)</td>
<td>78.08 ± 7.700</td>
<td>77.80 ± 13.80</td>
<td>78.8 ± 3.40</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>5.720 ± 0.150</td>
<td>5.830 ± 0.280</td>
<td>5.92 ± 0.13</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>14.98 ± 0.100</td>
<td>14.88 ± 0.120</td>
<td>15.5 ± 0.17</td>
</tr>
<tr>
<td>PCT (%)</td>
<td>0.043 ± 0.004</td>
<td>0.039 ± 0.009</td>
<td>0.041 ± 0.003</td>
</tr>
</tbody>
</table>

a, b and c mean significantly different from control, ENRO-A and ENRO-B, respectively; (P<0.05).

Table 2: Comparative Effects of Oral Administration of 10 mg/Kg Body Weight Per Day for 5 Consecutive Days of ENRO-A and ENRO-B on the Erythrocytary Parameters in Broiler Chickens (Mean ± SE; N=6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ENRO-A</th>
<th>ENRO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (x10^9/L)</td>
<td>2.600 ± 0.07</td>
<td>2.500 ± 0.08</td>
<td>2.700 ± 0.14</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>14.75 ± 0.50</td>
<td>14.10 ± 0.60</td>
<td>13.95 ± 0.40</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>31.80 ± 0.90</td>
<td>29.80 ± 1.30</td>
<td>30.50 ± 1.40</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>119.2 ± 1.50</td>
<td>117.9 ± 1.60</td>
<td>115.3 ± 2.00</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>54.90 ± 1.40</td>
<td>55.80 ± 1.20</td>
<td>53.05 ± 2.65</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>46.10 ± 0.65</td>
<td>48.10 ± 0.99</td>
<td>45.98 ± 1.67</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>8.300 ± 0.40</td>
<td>7.800 ± 0.30</td>
<td>8.500 ± 0.60</td>
</tr>
</tbody>
</table>

a, b and c mean significantly different from control, ENRO-A and ENRO-B, respectively; (P<0.05).
As shown in Table 4, ENRO-A and ENRO-B significantly (P<0.05) increased blood glucose and triglyceride levels compared to that of control. Cholesterol level was increased significantly (P<0.05) only after ENRO-B repeated administration. However, other metabolic parameters showed insignificant changes.

Table 3: Comparative Effects of Oral Administration of 10 mg/Kg Body Weight Per Day for 5 Consecutive Days of ENRO-A and ENRO-B on Selected Biochemical Parameters Related to Liver, Heart and Kidney Functions in Broiler Chickens (Mean ± SE; N=6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ENRO-A</th>
<th>ENRO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>34.56 ± 4.8</td>
<td>45.23 ± 5.6</td>
<td>40.53 ± 4.5</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>588.05 ± 75.3</td>
<td>743.05 ± 92.3</td>
<td>665.45 ± 73.0</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>166.2 ± 21.1</td>
<td>187.25 ± 24.3</td>
<td>176.75 ± 18.5</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>112.65 ± 14.8</td>
<td>336.25 ± 26.6</td>
<td>185.35 ± 0.8</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>6.61 ± 0.1</td>
<td>9.00 ± 0.4</td>
<td>0.85 ± 0.1</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>14.48 ± 2.8</td>
<td>26.6 ± 0.4</td>
<td>23.40 ± 0.4</td>
</tr>
</tbody>
</table>

Table 4: Comparative Effects of Oral Administration of 10 mg/Kg Body Weight Per Day for 5 Consecutive Days of ENRO-A and ENRO-B on Selected Biochemical Parameters Related to Metabolism in Broiler Chickens (Mean ± SE; N=6).

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>ENRO-A</th>
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<tr>
<td>TP (mg/dL)</td>
<td>4.88 ± 0.2</td>
<td>7.8 ± 0.3</td>
<td>4.53 ± 0.5</td>
</tr>
<tr>
<td>AL (mg/dL)</td>
<td>1.82 ± 0.0</td>
<td>13.3 ± 0.6</td>
<td>18.7 ± 0.2</td>
</tr>
<tr>
<td>GLs (mg/dL)</td>
<td>2.81 ± 0.0</td>
<td>29.3 ± 0.6</td>
<td>15.55 ± 0.2</td>
</tr>
<tr>
<td>TAGs (mg/dL)</td>
<td>82.46 ± 4.9</td>
<td>26.91 ± 0.4</td>
<td>0.85 ± 0.1</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>91.08 ± 9.0</td>
<td>103.99 ± 12.4</td>
<td>0.85 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>218.55 ± 7.9</td>
<td>257.25 ± 9.3</td>
<td>0.85 ± 0.1</td>
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a, b and c mean significantly different from control, ENRO-A and ENRO-B, respectively; (P<0.05).

3.3. Histopathological findings

Table There were no histopathological alterations and normal histological structures of the central and portal veins with surrounding hepatocytes were recorded in the liver of control group (Fig. 2 & 3). Oral administration of enrofloxacin 10% (Brand A) daily at a dose of 10 mg/kg body weight for five consecutive caused moderate focal lymphoid cells aggregation was observed in the hepatic parenchyma (Fig. 4) and dilatation in the portal vein associated with inflammatory cells infiltration mainly lymphocytes in the portal area (Fig. 5) while in ENRO-B, dilatation of the central vein was noticed (Fig. 6); and the hepatic parenchyma showed severe focal aggregation of the lymphoid cells (Fig. 7).

Table 4: Comparative Effects of Oral Administration of 10 mg/Kg Body Weight Per Day for 5 Consecutive Days of ENRO-A and ENRO-B on Selected Biochemical Parameters Related to Metabolism in Broiler Chickens (Mean ± SE; N=6).

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Kidneys dissected from control group, ENRO-A- or ENRO-B-treated groups showed no histopathological alterations and the normal histological structure of the glomeruli and tubules were recorded in figures 8, 9 & 10, respectively. Oral administration of ENRO-A caused mild focal inflammatory cells infiltration in between the myocardial bundles (Fig. 11) on the other hand, control and ENRO-B groups showed normal histological structure of the myocardial bundles (Figs. 13 & 14), respectively, with reference to control (Fig. 12).

4. Discussion

The 2nd generation fluorquinolones were developed in the 1980s, exhibited increased antibacterial activity by the Enterobacteriaceae and other Gram-negative bacteria (such as P. aeruginosa), and had some activity against certain Gram-positive cocci in addition. Structural changes associated with the 2nd generation, from the 1st one, increased their oral bioavailability and systemic distribution. They include norfloxacin, ciprofloxacin, enrofloxacin, danofloxacin, difloxacin and marbofloxacin.
Among these, enrofloxacin (Fig. 1) was developed exclusively for veterinary use in many animal species including, cattle, pigs, dogs, cats… etc. (Altreuther 1987); and for the treatment of respiratory disease in chickens and turkeys (Anderson et al. 2003). After administration, ENRO is partly de-ethylated to ciprofloxacin in vivo, which is also pharmacologically active and is employed in human medicine (Rao et al. 2002). The drug is being used in many developing countries as a routine choice to treat almost any bacte-
rial disease in poultry, although its use has been banned in some advanced countries (Sumano et al. 2003). Bioequivalence studies are generally recommended by FDA using the following endpoints: Pharmacokinetic endpoint; Pharmacody-
namic endpoint; Clinical endpoint; and in vitro endpoint (Midha and McKay 2009). The authors evaluated the same tested ENRO brands from the pharmacokinetic endpoint in a previous study (Ehmeza et al. 2016). Here, the drug preparations were compared from the clinical point of view.

Estimation of plasma clinicochemical biomarkers, blood parameters and indices, as well as histopathological alterations may give a major picture about assessment of a drug safety. Liver and kidney, in particular, are target organs as they are the sites of drug metabolism and elimination. The heart function was also evaluated as the heart is a vital organ and could be affected by some drugs.

Repeated oral administration of ENRO-A induced a significant (p<0.05) increase of the activity of alkaline phosphatase compared to ENRO-B as well as control group (Table 3). While other hepatic biomarkers, ALT and AST remained insignificantly changed after ENRO-treatment.

Although ALP is formed mostly in the liver, yet, it is nonspecific to hepatic injury as it is formed by other tissues as bone, kidney, skeletal muscle and placentas. However, the degenerative changes in the liver tissue shown in figures (4 & 5) may give a support to the hypothesis of hepatic injury caused by ENRO-A administration. This adverse hepatic effect may be attributed to the enrofloxacin-induced reactive oxygen species generation as reported by Ibrahim and Yarsan (2009) and Sureshkumar et al. (2013b). The lesser changes recorded after ENRO-B administration (Figs. 6 & 7) may be attributed to the difference in pharmaceutical technologies adopted in ENRO brands industries. Oral administration may be stressful on the liver more than injection because of the first pass effect. It is also worthy to note that ALP concentration in juvenile birds is significantly higher than that of adult birds as it is induced by increased cellular activity and synthesis rather than cell damage. In contrast to mammals, ALP of birds is also found at higher concentrations in duodenum and kidney (Clubb et al. 1990). This finding may be not in accordance with that of Sureshkumar et al. (2013a) who showed that administration of enrofloxacin 10% daily at a dose of 10 mg/kg body weight for 5 consecutive days via drinking water to broiler chickens induced insignificant increase in levels of ALP. This difference may be attributed to different research environment and different ENRO source.

Although histopathological examination did not reveal obvious pathological changes in the kidney tissues, both tested brands of ENRO caused significant increases in the levels of plasma urea and creatinine concentrations compared to control (p<0.05), with higher significance in case of ENRO-A. Urea is formed in the liver and represents the principle end-product of protein catabolism. It is excreted almost entirely by the kidneys and about 25 to 40 % of the filtered urea is reabsorbed. Thus, the recorded high value in the present study may refer to hyper-ammonaemia, rather than uremia, that resulted from the adversely affected liver; and/or reduced glomerular filtration rate. Creatinine is a specific indicator for evaluating kidney function as it is excreted only via this route. However, inability to record obvious pathological changes in the renal tissue may attribute the recorded high concentrations of creatinine to either the decreased glomerular filtration rate because of extra-renal cause; and/or muscular damage where creatinine is the metabolite of the muscular creatine phosphate. The obtained result may be inconsistent with Sureshkumar et al. (2013a) who reported that administration of enrofloxacin 10% daily at a dose of 10 mg/kg body weight for 5 consecutive days via drinking water to broilers induced insignificant increase in the serum level of urea concentrations; but consistent with those of Küng et al. (1993) and Sureshkumar et al. (2013a) who reported significant increases in serum creatinine.

Creatine kinase (CK) is a dimeric enzyme occurring in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CK-BB (brain type) and CK-MB (myocardial type). The determination of CK and CK-isoenzyme activities is utilized in the diagnosis and monitoring of myocardial infarction and myopathies. Following injury to the myocardium, CK is released from the damaged myocardial cells. In early cases, a rise in the CK activity can be found just 4 hours after an injury, the CK-activities reaches a maximum after 12-24 hours and then falls back approximately to the normal range after 3-4 days. Elevated levels of CK reported in this study may indicate a possible mild damaging effect of ENRO-A, but not ENRO-B, on the myocardial cells of broilers that were treated repeatedly for 5 consecutive days. The result is parallel to the histopathological findings reported in the present study, where repeated oral administration of ENRO-A caused mild focal inflammatory cells infiltration in between the myocardial bundles (Fig. 13), but control and ENRO-B groups showed normal histological structure of the myocardial bundles (Figs. 12 & 14), respectively. The data may be partially consistent with that reported by Sureshkumar et al. (2013a) in chickens.

Glucose is the primary energy source for the animal body. It is derived from the breakdown of carbohydrates in diet and in body stores as well as by endogenous synthesis from protein or the glycerol moiety of triglycerides. Glucose level in blood is maintained within a fairly narrow range by regulatory hormones such as insulin, glucagon or epinephrine. Glucose is excreted in urine in minute undetectable amounts which in turn reabsorbed by renal tubules whatever it is within the normal range. As shown in table (4), both ENRO-A and ENRO-B significantly (p<0.05) increased blood glucose levels compared to that of control. This may be speculated as a result from the increased rate of glycogenolysis or gluconeogenesis by the liver and muscles and/or the decreased insulin secretion by the pancreatic islets. Glucose homeostasis at the recorded normal high levels in chickens remains as not yet understood physiological phenomenon. This finding may be not in accordance with that of Aziz (2005) who reported insignificant decline in levels of serum glucose in chicken treated with enrofloxacin 10 % for 5 consecutive days via drinking water. The present study showed that both ENRO brands at the given dose regimen to broilers caused significant increases in triglyceride levels. While, cholesterol significant increase was recorded after only ENRO-B administration. These abnormal changes in lipid parameters may follow hepatic dysfunction caused by ENRO repeated administration. This result may be inconsistent with the finding of Aziz (2005) who recorded insignificant decline in cholesterol level after enrofloxacin 10% oral solution administration. Protein parameters did not show significant changes after both ENRO brands treatment in broiler chickens. The finding may be consistent with that of Küng et al. (1993) reported that administration of enrofloxacin 20% daily at a dose of 10 mg/kg body weight for 5 consecutive days was accompanied with insignificant decrease in total proteins and globulins. Haematological profiles revealed significant increase in platelets distribution width (PDW) after oral administration of ENRO-B 10% solution daily at a dose of 10 mg/kg body weight for 5 consecutive compared with that of ENRO-A and control group (p<0.05). However, other haematological parameters did not show significant changes. A normal PDW indicates that platelets are mostly of the same size, while a high PDW means that platelet size varies greatly, a clue that there may be a disorder affecting platelets. A false result may be recorded by the virtue of clumping some platelets together in blood samples. Aziz (2005) stated that differences in hematological values between treated group with enrofloxacin 10% for 5 consecutive days via drinking water and corresponding control groups were not significant (p>0.05), giving almost accordance with our data. Ibrahim et al. (2011) found only significant increases in hemoglobin level and mean corpuscular hemoglobin (MCH) values when enrofloxacin 10% was orally administered at a dose of 10 mg/kg body weight daily for 5 consecutive days to chickens compared with corresponding controls (p<0.05). However, other haematological parameters including RBCs, PCV, MCHC, MCV, WBCs and the differential leukocyte
counts did not show any significant changes. With exception of Hb and MCH, these findings are in accordance with the recorded ones in the present study and suggest that administration of enrofloxacin 10% daily at a dose of 10 mg/kg body weight for 5 consecutive days do not cause anemia.

5. Conclusion

From the safety endpoint, any of the tested brands, Enrol® or Syv-aquinos® did not cause any clinical serious manifestations on the treated birds, yet laboratory analysis revealed some adverse effects on some organs as liver and heart as well as alterations in some clinicochemical parameters especially after ENRO-A Brand. The recorded side effects are a common feature of almost all drugs and usually disappear after completing the course of drug therapy.

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