

Pharmacovigilance of Tilmicosin in Mice

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ABSTRACT

Background: Tilmicosin is a macrolide antibiotic used mainly for controlling respiratory infections in animals. Tilmicosin therapy, like that with other macrolide members, may be associated with various adverse effects on different organ functions that remain a controversial matter. Therefore, the object of the present study was to investigate the adverse effects of tilmicosin, a relatively new macrolide, on biochemical and haematological parameters as well as histological structure in mice in a trial to evaluate its safety after administration of a small and larger doses subcutaneously.

Materials, Methods & Results: Forty male Swiss albino mice were assigned randomly to four groups; the first group was untreated while the other three were treated with escalating doses of tilmicosin (20, 40 & 60 mg/Kg, SC). Tilmicosin caused tender swellings at the site of injection and animals received a dose 60 mg/kg of body weight died shortly postadministration. Animals received smaller doses, 20 & 40 mg/kg of body weight survived and blood, plasma and tissue samples showed marked dose-dependent alterations. Tilmicosin significantly decreased all (but not MCV) erythrocytic parameters and indices including, RBC count, HCT, HGB, MCH and MCHC. Additionally, lymphocytes exhibited significant decrease, while granulocytes exhibited significant increase compared to control. Hepatorenal function markers including, ALP, ALT, AST, urea and creatinine showed significant increases, compared to control, particularly post-administrating 40 mg/kg of body weight dose. Cardiac function marker, CK, showed significant increase after both doses of tilmicosin. Significant decreases in TP, ALB and GLB concentrations compared to control; and, on contrary, significant increases in CHOL, TAG and GLU were also recorded. Parallel dose-dependent degenerative changes were also observed in liver, kidney, heart and spleen tissue samples picked from treated groups.

Discussion: The subnormal levels of erythrocytic parameters and indices recorded from treated groups in this study may indicate that tilmicosin administration caused acute anemia in a dose-dependent manner. Low number of RBCs and decreased percentage of its haematocrit value indicates bone marrow dysfunction that might be caused by tilmicosin in this study. Tilmicosin-induced renal dysfunction was proved in the present study indicated by high levels of creatinine and urea. Renal dysfunction may result in decreased erythropoietin production, a key hormone in RBC synthesis. Recorded effects of tilmicosin on leukocytic counts could be related to tilmicosin-induced "stress" of the treated animals. Increased ALP, ALT and AST activity from one hand and urea and creatinine from the other hand may indicate that tilmicosin at the tested doses caused significant changes in hepatic and renal tissues, respectively. Higher CK activity in the treated groups indicate cardiotoxicity of tilmicosin. Histopathological findings were parallel to the biochemical ones and supportive to them. Hepatorenal dysfunction caused what is well-known as "metabolic syndrome" that was associated with adverse effects on metabolic parameters towards the negative side in tilmicosin-treated mice. These data indicate that tilmicosin, especially in large but-tolerated doses, may cause adverse effects on blood and organ functions and structures; and thus particular care and monitoring programs should be followed if it is used in therapy.

Keywords: Biochemical, haematological, hepatorenal, safety, tilmicosin.

INTRODUCTION

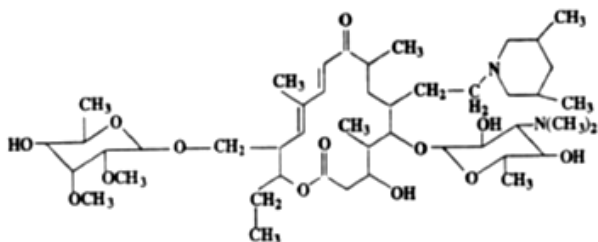
Tilmicosin is a bacteriostatic macrolide antibiotic working by invading the cell membrane of sensitive bacteria and binding to the 50s ribosome subunit, preventing protein synthesis; translocation between the 50s and 30s ribosomal subunits is interrupted, causing early detachment and thus creating of incomplete peptide chains [1]. Tilmicosin has been developed in an injectable form for use in cattle and sheep to treat respiratory infections (10 mg/kg bw); and as a feed premix for swine (200-400 mg/kg feed) for 10 to 21 days, equivalent to 8-20 mg/kg bw per day [26]. It is an effective antimicrobial for Gram-positive and some Gram-negative bacteria, as well as atypical bacteria as *Mycoplasma* spp.

Pharmacovigilance is defined as the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem. [32].

Treatment with tilmicosin, like with other drugs, may be associated with different adverse effects, which may include pharmacological, haematological, clinicochemical, pathological and others. Clinicochemical ones are generally considered to be indicators of pathological adverse effects [18]. Generally, macrolides may cause gastrointestinal upsets in addition to hepatotoxicity and jaundice as reported by [6]. However, tilmicosin in particular has different side effects from other macrolides, including cardiotoxicity [24], dyspnoea, anaphylaxis, collapse and death [6,13].

The objectives of this study were to determine the alterations that may follow tilmicosin subcutaneous administration in haematological, biochemical and histopathological parameters in albino mice as an experimental model.

MATERIALS AND METHODS

Tilmicosin

Tilmicosin is a macrolide antibiotic used to combat bacterial infections primarily respiratory ones. Its chemical name is 20-deoxo-20-(3,5-dimethyl piperidin-1-yl) desmycosin. It is structurally similar to tylosin, having the above chemical formula ($C_{46}H_{80}N_2O_{13}$) with molecular weight of 869.15. Tilmicosin is a mixture of one cis and two trans-isomers in the approximate ratio 85:15. Physically, it is freely soluble (1500 mg/L or greater) in organic solvents (hexane, acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, methanol, tetrahydrofuran); water solubility is temperature and pH dependent, but is 566 mg/mL at pH 7 and 25°C. Tilmicosin was obtained as the patent preparation Pneumotac^{®1} that is a subcutaneous therapy for pneumonia and other respiratory disease conditions in cattle and sheep, formulated as 100 mL amber glass vials containing 333.828 mg tilmicosin phosphate/mL, equivalent to 300 mg tilmicosin/mL. The drug solution was further diluted in sterile water to adjust dose volumes as 0.3 mL diluted solution equivalent to 20 (small dose) and 40 (large dose) mg/kg bw of mice.

Experimental animals

Forty male Swiss albino mice weighing 25-30 g were used for the present study. All animals were maintained on standard pellet diet and water *ad libitum*. This study protocol was approved by the Committee on the Ethics of Animal Experiments of Benha University. Blood sampling and organ dissection were performed under light ether anesthesia, and all efforts were made to minimize suffering were according to the local regulations of the use of experimental animals. Even though, the group of mice that received 60 mg/Kg body weight, have unexpectedly and rapidly (within time range 15~30 min) died. The authors could not, therefore, take any humane measures against the observed lethality until they naturally happened. The condition of the animals was monitored after injection continuously for 1 h to record any behavioural changes or abnormal symptoms; within this 1-h period, the drug should reach high concentration after being absorbed from its SC injection site. Afterwards, the animal were observed routinely every 6 h. The permission taken from "The Committee on Ethics of Animal Experiments" of undertaking the present study based upon that the selected doses were within the tolerable range according to literature.

Experimental design

A parallel design is followed in this experiment, where animals were randomly divided into four groups (n = 10 for each) and labelled appropriately. The first group received a single dose (equivalent to 20 mg/kg bw) of tilmicosin injectable solution subcutaneously in the scruff region. The second and the third groups received larger doses of tilmicosin (40 and 60 mg/kg bw, respectively) in the same manner. The fourth group received the same volume of the diluent (0.3 mL of sterile water, subcutaneously) and kept as control. After different treatments, all animals were observed daily for mortality and extraordinary symptoms throughout the period of study. Blood samples were collected from the retro-orbital venous plexus using heparinized capillary tubes 72 h post-injection. The samples were received into centrifuge tubes containing lithium heparin. Each sample was divided into two parts; the first one was kept as whole blood for haematological analysis, while the second part was centrifuged for 5 min at 12,000 g to separate plasma that was used for the biochemical study. Animals were then humanely sacrificed, and the liver, kidneys, heart and spleen were dissected out for the histopathological examination. Sampling did not involve mice of the third group as all of them died shortly after injection.

Haematological assays

To assess the blood safety profile of tilmicosin, the following haematological parameters were automatically evaluated by auto-haematology analyser (Model BC-2800Vet)². 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 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 autoanalyser one at a time as 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:

$$HCT(\%) = \frac{RBC(10^{12}/L) \times MCV(fL)}{10}$$

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

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

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

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

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

Where, PL = particles in the lymphocyte region (10⁹/L); PM = particles in the mid-size region (10⁹/L); PG = particles in the granulocyte region (10⁹/L).

PLT count (10⁹/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). PCT is derived from the following equation:

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

Clinicochemical assays

Estimating plasma biochemical parameters, was carried out spectrophotometrically³ using diagnostic kits⁴. The parameters were alkaline phosphatase (ALP) [10], aspartate aminotransferase (AST) [9], alanine aminotransferase (ALT) [9], total protein (TP) [30], creatine kinase (CK) [11], albumin (ALB) and globulin (GLB) [21], urea [19], creatinine [7], glucose (GLU) [29], total cholesterol (CHOL) [2]. Plasma triacylglycerols (TAG) were estimated using a diagnostic kit purchased from Biolabo SA (Maizy, France) according to Fossati and Prencipe [15].

Histopathological assay

The liver, two kidneys, spleen and heart were taken from sacrificed mice in different groups and preserved in formalin solution 10% and subjected for microscopical examination according to Bancroft and Gamble [5].

Preparation of the samples: Samples were left for fixation in 10% neutral formalin for twenty four hours. Then they were washed by running water over night. The washed samples were dehydrated by using graded increased concentrations of ethyl alcohol starting with 70% and ending with absolute alcohol. The dehydrated samples were immersed in xylol for 3 h till clearance and then embedded in melted paraffin wax that was left to solidify after tissue immersion. Thin sections (4 - 6 μ m) were prepared from the solidified paraffin blocks by a rotative microtome⁵.

Procedure of staining and examination: Paraffin was removed from the sections by two changes of absolute alcohol (5 min in each) which was removed by washing with tap water. Sections were stained with Harris haematoxylin and eosin for 10 min, and then washed with running water for 15 min. Stained samples were then dehydrated by different concentrations of alcohol, and then immersed in xylol for clearance and covered by DBX. The obtained slides were subjected for routine microscopical examination.

Statistical analysis

Results are expressed as mean \pm standard error of the mean of 10 observations (n). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA). *P*-values of 0.05 or less were considered significant. All statistical analytical procedures were done using SPSS software.

RESULTS

Abnormal localized tender swellings were developed after subcutaneous injection of all tested doses of tilmicosin. Abnormal signs of toxicity including drowsiness, ataxia, dyspnoea, gasping followed by death were recorded after 15-30 min of treatment of mice with the dose of 60 mg/kg body weight and above. No samples were taken from these animals and thus data could not be recorded. Animals received tilmicosin 20 (small dose, SD) and 40 (large dose, LD) mg/kg of body weight showed no signs of toxicity and remained alive and analysis of samples taken from them showed the following results.

Haematological study

The administration of tilmicosin significantly and dose dependently affected both the erythrocyte and leukocyte parameters compared to the control group (*P* > 0.05). Small dose of tilmicosin (2nd group), significantly decreased HGB, MCH and MCHC values. Other erythrocytic parameters were not significantly affected by the injected small dose of tilmicosin.

Larger dose of tilmicosin (3rd group), significantly decreased all erythrocytic parameters and indices (except MCV) including, RBC count, HCT, HGB, MCH and MCHC. Both tested doses of tilmicosin had no significant effects on almost all of leukocytic parameters except lymphocytes which exhibited significant decrease and granulocytes which exhibited significant increase compared to control, only after administration of the large dose of tilmicosin (Tables 1 & 2).

Table 1. Effects of subcutaneous injections of tilmicosin (20 & 40 mg/kg of body weight) on erythrocytic parameters of mice (Mean \pm SE; *n* = 10).

Group/ Parameter	Control	Tilmicosin SD	Tilmicosin LD
RBC (10 ¹² /L)	8.10 \pm 0.29	7.42 \pm 0.33	6.07 \pm 0.28*
HCT (%)	40.87 \pm 1.11	37.83 \pm 1.71	32.83 \pm 1.75*
HGB (g/dL)	13.67 \pm 0.58	11.27 \pm 0.32*	9.25 \pm 0.46*
MCV (fL)	50.49 \pm 0.70	51.01 \pm 1.79	54.17 \pm 1.99
MCH (pg)	16.83 \pm 0.12	15.27 \pm 0.55*	15.24 \pm 0.25*
MCHC (g/dL)	33.41 \pm 0.72	29.82 \pm 0.48*	28.19 \pm 0.62*

*Significantly different from Control (*P* < 0.05).

Table 2. Effects of subcutaneous injections of tilmicosin (20 & 40 mg/kg of body weight) on leukocytic parameters of mice (Mean \pm SE; $n = 10$).

Group/Parameter	Control	Tilmicosin SD	Tilmicosin LD
WBC ($10^9/L$)	7.5 \pm 0.84	6.9 \pm 0.78	6.33 \pm 0.72
Lymphocyte (%)	43.29 \pm 2.91	39.48 \pm 3.58	29.59 \pm 4.96*
Mid-sized cells (%)	17.89 \pm 1.0	16.48 \pm 1.95	13.85 \pm 0.93
Granulocytes (%)	38.82 \pm 3.60	48.03 \pm 3.33	56.56 \pm 4.79*
PLT ($10^9/L$)	0.652 \pm 0.072	0.681 \pm 0.059	0.705 \pm 0.047
PCT (%)	0.35 \pm 0.04	0.38 \pm 0.07	0.404 \pm 0.05
MPV (fL)	5.40 \pm 0.10	5.57 \pm 0.18	5.73 \pm 0.15

*Significantly different from Control ($P < 0.05$).

Clinicochemical study

The activity of alkaline phosphatase did not exhibit a significant change after administration of the small dose of tilmicosin; but a significant increase in its activity was observed after administration of the larger dose. Other hepatorenal function markers including, ALT, AST, urea and creatinine showed significant increases, compared to control, in the two dose levels studied. Additionally, cardiac function marker,

CK, showed significant increase after both doses of tilmicosin (Table 3).

The small dose of tilmicosin significantly increased cholesterol level compared to control; yet, no significant changes were observed on other metabolic parameters. Larger dose of tilmicosin caused, however, significant decreases in TP, ALB and GLB concentrations compared to control; and, on contrary, caused significant increases in CHOL, TAG and GLU (Table 4).

Table 3. Effects of subcutaneous injections of tilmicosin (20 & 40 mg/kg of body weight) on liver and renal function parameters of mice (Mean \pm SE; $n = 10$).

Group/Parameter	Control	Tilmicosin SD	Tilmicosin LD
ALP (IU/L)	204.27 \pm 13.27	320.01 \pm 61.61	671.07 \pm 53.51*
AST (IU/L)	23.27 \pm 1.79	113.37 \pm 13.01*	140.28 \pm 10.71*
ALT (IU/L)	13.43 \pm 3.14	36.70 \pm 2.88*	95.47 \pm 15.79*
CK (IU/L)	114.30 \pm 7.59	185.85 \pm 11.92*	416.20 \pm 19.09*
Urea (mg/dL)	49.65 \pm 15.56	108.96 \pm 18.53*	168.31 \pm 16.76*
Creatinine (mg/dL)	1.65 \pm 0.26	2.41 \pm 0.52*	3.77 \pm 0.61*

*Significantly different from Control ($P < 0.05$).**Table 4.** Effects of subcutaneous injections of tilmicosin (20 & 40 mg/kg of body weight) on some metabolic parameters of mice (Mean \pm SE; $n = 10$).

Group/Parameter	Control	Tilmicosin SD	Tilmicosin LD
TP (g/dL)	5.16 \pm 0.14	4.8 \pm 0.1	3.31 \pm 0.41*
ALB (g/dL)	1.72 \pm 0.04	1.69 \pm 0.04	1.25 \pm 0.22*
GLB (g/dL)	3.43 \pm 0.16	3.10 \pm 0.16	2.06 \pm 0.20*
CHOL (mg/dL)	66.15 \pm 6.01	177.26 \pm 9.31*	225.32 \pm 11.57*
TAG (mg/dL)	53.48 \pm 6.74	78.56 \pm 8.87	125.64 \pm 8.01*
GLU (mg/dL)	93.43 \pm 19.16	108.76 \pm 6.52	139.28 \pm 8.22*

*Significantly different from Control ($P < 0.05$).

Histopathological study

Liver of control group exhibited no histopathological alterations and the normal histological structure of the central vein and surrounding hepatocytes was recorded (Figure 1a). Liver of mice treated with a small dose of tilmicosin showed mild dilatation in the central veins associated with diffuse kupffer cells proliferation in between the hepatocytes (Figure 1b). Meanwhile large dose of tilmicosin caused focal necrosis of hepatic parenchyma associated with dilatation of the central veins (Figure 1c).

There was no histopathological alteration in the kidney of the control group mice and the normal histological structure of the glomeruli and tubules at the cortex were recorded (Figure 2a). Focal inflammatory cell infiltration was detected in between the glomeruli and tubules at the cortex of the kidney of mice received small dose of tilmicosin (Figure 2b). Larger dose of tilmicosin caused focal inflammatory

cell infiltration in between the tubules and surrounding the congested blood vessels, associated with appearance of homogenous eosinophilic casts in the lumen of cystically dilated tubules (Figure 2c).

There was no histopathological alteration in the heart of control group mice and the normal histological structure of the myocardial muscle bundles was recorded (Figure 3a). There was degeneration and necrosis of cardiac cells and cardiac muscle fibers of mice treated with a small and large doses of tilmicosin as recorded in (Figure 3b) and (Figure 3c).

There was no histopathological alteration in spleen of mice belonging to group 1 and the normal histological structure of the white and red pulps was recorded (Figure 4a). Megakaryoblasts were observed in diffuse manner all over the splenic tissue in both pulps after treating mice with a small and large doses of tilmicosin (Figures 4b and 4c).

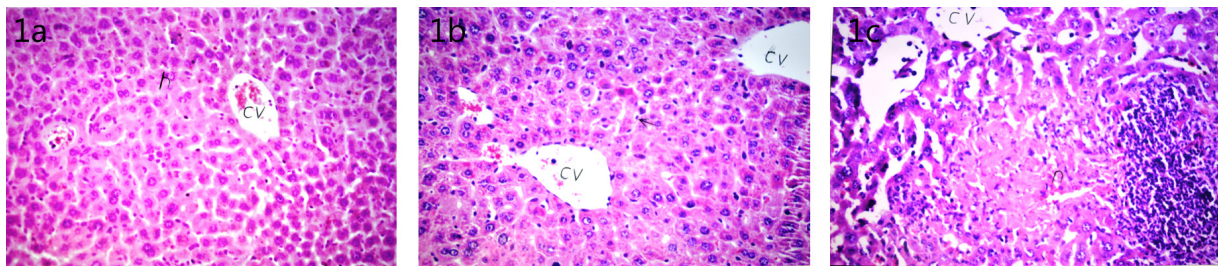


Figure 1. Histological section of liver of a control mouse showing normal hepatic structure of central vein and surrounding hepatocytes (1a); liver of 20 mg tilmicosin/kg of body weight treated mouse showing mild dilatation of the central vein with diffuse Kupffer cells proliferation between hepatocytes (1b); and liver of 40 mg tilmicosin/kg of body weight treated mouse showing focal necrosis in the hepatic parenchyma with dilatation of the central vein (1c) [H&E x40].

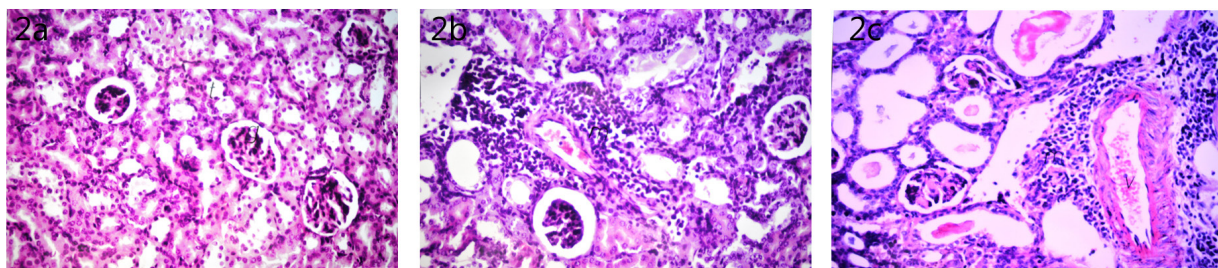


Figure 2. Histological section of kidney of a control mouse showing normal structure of the glomeruli and tubules at the renal cortex (2a); kidney of 20 mg tilmicosin/kg of body weight treated mouse showing focal inflammatory cell infiltration in between glomeruli and tubules at the cortex (2b); and kidney of 40 mg tilmicosin/kg of body weight treated mouse showing congestion of blood vessels with focal inflammatory cell infiltration in between glomeruli and tubules (2c) [H&E x40].

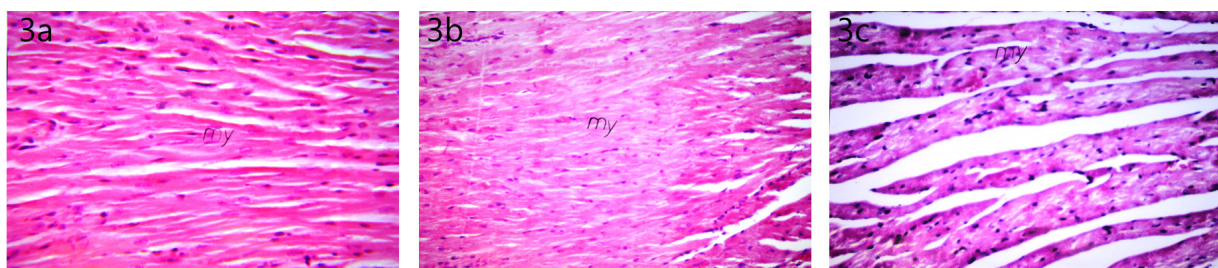


Figure 3. Histological section of heart of a control mouse showing normal structure of the myocardial bundles (3a); heart of 20 mg tilmicosin/kg of body weight treated mouse showing focal necrotic foci in the myocardium (3b); and heart of 40 mg tilmicosin/kg of body weight treated mouse showing necrotic focal and breakage areas in the myocardium (3c) [H&E x40].

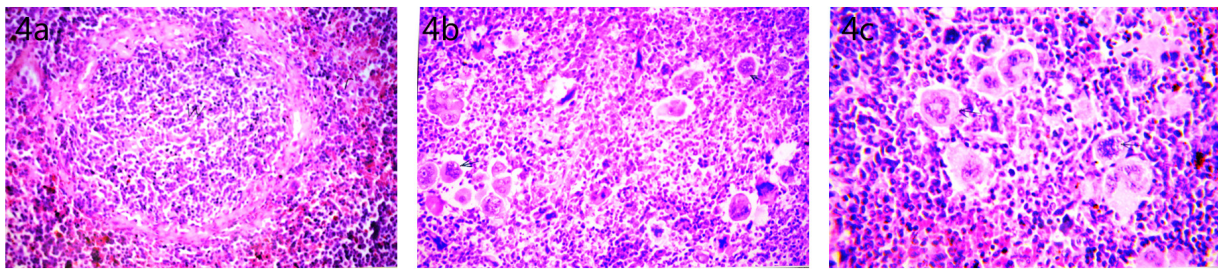


Figure 4. Histological section of spleen of a control mouse showing normal structure of the lymphoid cells in the white pulp and the adjacent red pulps (4a); spleen of 20 and 40 mg tilmicosin/kg of body weight treated mouse showing multiples of megakaryoblasts in a diffuse manner (4b & 4c) [H&E x40].

DISCUSSION

The macrolide antibiotic tilmicosin is being used in treatment of respiratory diseases in different animal species including cattle [26], horse [33], swine [23], sheep [13], goat [28], rabbit [25] and turkey [16]. Therapy with macrolides is sometimes associated with gastrointestinal disturbances, jaundice, and transient swelling at injection site [6]. Furthermore, tilmicosin particularly was reported to cause cardiotoxicity [17]. Pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems. WHO established its Programme for International Drug Monitoring in response to the thalidomide disaster detected in 1961. At the end of 2010, 134 countries were part of the WHO PV Programme. The aims of PV are to enhance patient care and patient safety in relation to the use of medicines; and to support public health programmes by providing reliable, balanced information for the effective assessment of the risk-benefit profile of medicines. The specific aims of pharmacovigilance are to improve patient care and safety in relation to the use of medicines and all medical and paramedical interventions, to improve public health and safety in relation to the use of medicines, to contribute to the assessment of benefit, harm, effectiveness and risk of medicines, encouraging their safe, rational and more effective (including cost-effective) use, and to promote understanding, education and clinical training in pharmacovigilance and its effective communication to the public [31].

The reported cardiotoxicity of tilmicosin pushed us to design this study as a trial to assess the various and common safety parameters that may be altered by pharmacological intervention including hematotoxicity, clinicochemical toxicity and histotoxicity.

The subnormal levels of RBC parameters and indices (measures of number, size and hemoglobin

concentration) calculated by automated cell counter in this study indicate that tilmicosin administration caused acute anemia in a dose-dependent manner. Low number of RBCs and decreased percentage of its haematocrit value indicates bone marrow dysfunction and/or blood loss that might be caused by tilmicosin in this study. Bone marrow dysfunction has been reported to be induced by drugs rather than tilmicosin including chloramphenicol, amphotericin, sulphonamides, phenylbutazone, novalgine and others [8] but there is no information about such adverse effect for tilmicosin. Further investigations are needed to confirm this speculation. RBC size is expressed by the mean corpuscular volume (MCV) in femtoliters (fL) and usually reflects the degree of regeneration from anemia. Macrocytosis (an increase in the MCV) usually correlates with a regenerative anemia, i.e. the bone marrow is reactive to repair the occurred decrease in blood parameters. Microcytic RBCs are the hallmark of iron-deficiency anemia [8]. MCV in the present study was insignificantly changed indicating normocytic anemia that might occur due to bone marrow dysfunction (hypoplastic anemia) and could be associated with renal failure and/or to endocrine disturbances. Tilmicosin-induced renal dysfunction was proved in the present study indicated by high levels of creatinine and urea. Renal dysfunction may result in decreased erythropoietin production, a key hormone in RBC synthesis. Normal MCV value may indicate additionally that tilmicosin-induced anemia is a non-regenerative type. The present data may be not in accordance with Oztekin *et al.* [27] and Altunok *et al.* [3] who reported that tilmicosin caused insignificant changes in erythrocytic parameters in mice and rabbits, respectively.

Regarding leucocytic parameters, large dose of tilmicosin caused significant decrease in lymphocyte percentage (lymphopenia) and significant increase in granulocyte percentage (granulocytosis). These abnor-

mal values occur due to many reasons. That is related to tilmicosin may be “stress” of the treated animals. Tilmicosin was proved in previous studies to cause oxidative stress in mice [4,34], rats [12] and chicks [14].

The estimation of some biochemical parameters such as the activities of enzymes in blood, tissues and body fluids plays a major role in assessment of a drug safety. The biochemical parameters in the tested groups (2nd and 3rd) in the present study were significantly different from those of control group. ALP, ALT and AST activity values at tested doses of tilmicosin were significantly increased compared to those of the controls. ALT is a cytoplasmic enzyme and its increased level in plasma is an indication of mild injuries caused by the drug to the liver. While AST is a mitochondrial enzyme whose increased activity in plasma reflects severe hepatic tissue injury [22]. It should be noted that 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 and placenta. Nevertheless, its increase along with AST and ALT may refer to that its source of elevation is hepatic. Degenerative changes in the liver tissue showed in figures 1b and 1c is confirmatory to this statement.

The recorded dose-dependent significant increase in urea and creatinine values at the tested doses of tilmicosin, suggests that the drug at these dose levels would cause renal damage. This biochemical result is confirmatory to the histopathological alterations recorded in kidney samples taken from the tested groups (Figures 2b and 2c). As urea is metabolized only in the liver, so its elevation also indicates hepatic dysfunction that was indicated above.

Elevated levels of CK in the present study indicates cardiotoxicity of tilmicosin that was well covered by previous studies [12,17]. The data are supported by degenerative changes including necrosis and breaks of myofibrils of myocardium (Figure 3c).

Hypoproteinemia is an established finding in liver damage [20] as the liver is the only factory of

plasma proteins. In the present study, the total plasma proteins including albumin and globulins concentrations were significantly decreased at all dose levels of tilmicosin tested. These findings further indicate the hepatotoxicity caused by the drug.

Parallel elevation of CHOL, TAG and GLU is also a sequel after liver injury, as these lipid and carbohydrate products are managed by the liver to keep them usually at physiological limits. Blood should be routinely cleared from supra-normal levels of CHOL and TAG by a healthy liver with the help of lipoproteins and lipase. Where, CHOL is utilized in the synthesis of steroidal hormones and TAG are stored in fat cells for energy when needed. GLU should be converted to glycogen by the liver and muscles as well. A diseased liver, thus, results in what is called “Metabolic Syndrome” that is indicated by elevated CHOL, TAG and GLU.

It is therefore expedient to advise that caution should be exercised in the use of tilmicosin in therapy to avoid high dose-induced adverse effects and its potential toxicity reported in the present study.

CONCLUSION

Results of the present data indicate that tilmicosin, particularly at high dose level, may be hepatotoxic, nephrotoxic and myelotoxic in mice; and thus it should be used with care in therapy.

MANUFACTURERS

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Ethical approval. All procedures, treatments and animal care were in compliance with the guidelines of the Committee on the Ethics of Animal Experiments of Benha University.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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