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Age and sex dependent side effects of tilmicosin on cardiac enzymes and hematological parameters in calves

Ahmed M Hegazy[1], Hazem M. Shaheen[1], Yaser H. A. Elewa[2, 3, *], Ismail Aboghaneima[4], Lamiaa Wasef[1], Gaber E. Batiha[1]

1Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhur 22511, Egypt; (A.M.H.), (H.M.Sh.), (L.W.), (G.E.B.);

2Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt; (Y.H.A.E.)

3 Laboratory of Anatomy, Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido 060-0818, Japan

4 Department of Physiology, Faculty of Veterinary Medicine, Damanhour University, Damanhur 22511, Egypt;

Abstract:

Tilmicosin is a macrolide antibiotic that is used mainly in veterinary medicine. Increased dose of tilmicosin lead to fatal cardiotoxic effects in humans. However, dose, age and sex dependent effect of tilmicosin in animals has not been identified yet. Therefore, we compared the side effect of two subcutaneous injection doses of tilmicosin on both cardiac enzymes (as indices of cardiac toxicity), and hematological parameters in calves among both sexes receiving at age of 6 and 12 months with that of control group. twenty-four calves were divided into eight groups (3 calves/each); two groups of 6-month male, two groups of 6-month female, two groups of 12-month male, two groups of 12-month female (each group receive twice subcutaneous doses "at day 1 and 4 of experiment" of either saline "control group" or tilmicosin "treated group"). Serum and blood were collected for both biochemical and hematological analysis among studied groups. In comparison to the control groups, the treated groups specially in female revealed significant increase of serum LDH and CK-MB (cardiac injury indicators), and granulocyte count, with significant decrease in the HCT, WBC, RBCs count. However, there was no significant difference among treated and control groups for the values of MID, MCV, HGB, lymphocytes and platelets count. Increased dose of tilmicosin could induce some side effects on hematological and biochemical parameters in calves and our study suggests sex differences in this effect.

Keywords: Blood parameters; cardiac enzymes; dairy calves; tilmicosin

*Correspondence: **Yaser H. A. Elewa[2, 3, *]**
Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.
Email: yaserelewa@yahoo.com

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1. Introduction

Antibiotics are classified into bacteriostatic and bactericidal that are widely used for the treatment of infectious diseases caused by bacteria (Wang, Yang, Shi, Song, & Yu, 2020). However, the unrestricted use of the antibiotics could lead to antibiotic resistance (Ventola, 2015), and or abuse concentrations that lead to residual in various body tissue specially in consumable farm animals and poultry. Furthermore, it could lead to some side

effects that range from mild symptoms, to mutagenic, teratogenic, and carcinogenic effects (Elsayed, Elkomy, Aboubakr, & Morad, 2014).

Macrolides are widely used antibiotics which first isolated in 1950 from soil bacteria (genus *Streptomyces*) and its mode of action is by binding to bacterial 50S ribosomal subunit and interfering with protein synthesis (Bhattacharjee, 2022). They showed high effectiveness against *Mycoplasma* spp. and Gram-positive organisms (including *Streptococcus* spp. and *Staphylococcus* spp.), but have only limited effectiveness against Gram-negative bacteria (Omura, 2002; Van Der Pol, Nelson, & Practice, 2016).

Since the discovery of erythromycin (the progenitor macrolide) in 1950, several generations of macrolides have been created to enhance bioavailability and pharmacokinetics. To combat the antibiotic resistance that has grown through time, such generations included the second generation of macrolides (such as clarithromycin and azithromycin), followed by a third generation of macrolides. However, it was found that these gains came with a number of major adverse effects (Dinos, 2017).

Tilmicosin is a novel broad-spectrum bacteriostatic macrolide antibiotic (Shryock, White, Staples, & Werner, 1996). Actinobacillus, Mannheimia haemolytica, Pasteurella, and several Gram-negative respiratory pathogens as well as various mycoplasma species have been found to be the main targets of tilmicosin's spectrum action. (Cockcroft, 2015; Ose, 1987; Prescott, Baggot, & Walker, 2000). It is synthesized from tylosin and is used only as veterinary antibiotic whereas it causes significant human toxicity at similar doses used in animals (Cockcroft, 2015; Oakes & Seifert, 2008). In poultry, it has been effectively used for the treatment of respiratory tract infections in poultry caused by *Mycoplasma synoviae*, *Mycoplasma gallisepticum*, *Pasteurella multocida*, and *Ornithobacterium rhinotracheale* (Nhung, Chansiripornchai, & Carrique-Mas, 2017). Furthermore, it is the drug of choice for treatment of the infections respiratory disease in cattle (Zeineldin, Lowe, & Aldridge, 2020). However, like other therapeutic drugs, the use of macrolides, particularly tilmicosin, has been associated with a number of side effects. Animal studies have shown that the use of tilmicosin can occasionally cause anaphylaxis, dyspnea, collapse, and even death (Croft et al., 2000; Gheith, El-Mahmoudy, Elmajdoub, & Awidat, 2015). Moreover, cardiovascular toxicity has been observed in human following unintentional or accidental exposures to tilmicosin (Forrester, 2005; McGuigan, 1994). However, no information about the adverse effect "in particular cardiotoxicity" of different doses of tilmicosin in cattle has been elucidated.

One of the cardiac enzymes that is vital to the metabolism of cellular energy is the creatine kinase (CK). It facilitates the reversible phosphorylation of ATP to create creatine phosphate from creatine. (Hettling & van Beek, 2011). Interestingly, creatine kinase- myocardial band (CK-MB) "an isoenzyme of the CK" is used as an indicator of cardiac myocyte damage (Bodor, 2016). Additionally, it has been observed that the risk of cardiovascular disorders is correlated with the serum level of lactate dehydrogenase (LDH) (Zhu et al., 2022). Therefore, the aim of the present study was to investigate the biochemical (CKMB and LDH) and hematological parameters in calves following administration of two doses of tilmicosin subcutaneously (s.c.) at both sexes and at age of 6 and 12 months in comparison with control groups, as well as among the two doses in the same group in a trial to evaluate its safety among different ages and sexes.

2. Materials and methods

2.1. Drug (Tilmicosin)

Tilmicosin was purchased from (Egyptian veterinary clinics) Company for veterinary products (Egypt) with a trade name (micotil 30% long-acting macrolide antibiotic) and administered as s.c. injection at dose of 10 mg/kg) / 150 kg bwt (Modric, Modric, Murphy, Bright, & Shults, 2011).

2.2. Experimental animals

Twenty-four healthy calves of both sexes (at age of six and twelve months with average weight of 150 and 300 kg, respectively) were obtained from a private dairy farm in Nubaria, Elbehera, Egypt. The calves were kept for seven days acclimatization to 12-hour light / dark cycle, and under controlled temperature of 21±2 °C. A standardized diet was given to animals besides water *ad libitum* throughout the experimental time.

All experiments were conducted in accordance with the standard Guidelines of Faculty of Veterinary Medicine, Damanhour University under all hygienic conditions using recoded published methods (Approval Number: DMU/VetMed-2025/037).

2.3. Treatments and experimental animals, design

The calves were allocated into two main groups; treated and control groups. The treated group received twice s.c. injections (at day 1 and 4) of 5ml tilmicosin. The calves in the saline were twice injected s.c. with 5ml buffered saline/ 150 kg bwt (at day 1 and 4). Each group is subdivided into four equal groups (3 calves/group) as shown below

Summary of doses and sexes among treated and control groups

Group 1 Three females, 6 months, 150 kg bwt Tilmicosin treated (10mg /kg bwt) 5 ml (1.5 gm.)/animal s.c

Group 2 Three females, 6 months, 150 kg bwt Buffered saline 5 ml s.c control

Group 3 Three males, 6 months, 150 kg bwt Tilmicosin Treated (10mg /kg bwt) 5 ml (1.5 gm.)/animal s.c

Group 4 Three males, 6 months, 150 kg bwt Buffered saline 5 ml s.c Control

Group 5 Three females, 12 months, 300 kg bwt Tilmicosin Treated (10mg /kg bwt) 10 ml (1.5 gm.)/animal s.c

Group 6 Three females, 12 months, 300 kg bwt Buffered saline 10 ml s.c Control

Group 7 Three males, 12 months, 300 kg bwt Tilmicosin Treated (10mg /kg bwt) 10 ml (1.5 gm.)/animal s.c

Group 8 Three males, 12 months, 300 kg bwt Buffered saline 10 ml s.c

2.4. Blood Sampling

Blood samples (5 mL) were collected from the calves of different groups on days 1, 2, 3, 4, 5, and 6 following the first s.c. injection and divided into two parts for hematological and biochemical examination. The first part (2ml) of blood samples from different groups were collected with EDTA and used for hematological analysis of both erythrocytes and Leukocyte parameters. And the other (3mL) of blood sample was centrifuged at 3000 r.p.m. for 10 minutes to obtain sera and the sera were preserved at – 30 °C for further biochemical examination. All calves were kept for one week after blood sampling to be monitored for cardiac toxicity signs.

2.5. Biochemical Analysis

The determination of serum cardiac enzymes (CK-MB, and LDH) among the studied groups was performed by using ready-made kits from Bio-Diagnostics Firm, Cairo, Egypt.

2.6. Hematological Analysis

Erythrocytes parameters include (red blood cell "RBC" count, hematocrit value "HCT", mean corpuscular volume "MCV", hemoglobin concentration "HGB"). The Leukocyte parameters include white blood cell (WBC) count, (MID) (Mid-Range Absolute Count), Granulocytes, lymphocytes, and platelets' (PLT) count.

2.7. Statistical analysis

The statistical analyses were carried out using (SPSS / PC). Quantitative data was expressed as median± standard deviation. One-way analysis of variance (ANOVA) test was performed for analysis of the differences between experimental groups followed by Duncan test (post-hoc test) for multiple comparisons among the experimental groups when significant differences were observed between studied groups. Statistically significant values were found when P value <0.05.

Results

3.1. Biochemical analysis

The serum level of both CK-MB, and LDH were compared among different groups as well as among the frst and second injection of the same group.

3.1.1. Serum level of CK-MB

The serum level of CK-MB among studied groups revealed a non-significant increase in CK-MB in the treated group than the control groups. However, sex difference was observed among studied female groups whereas a significant increase in the serum level of CK-MB was observed in the female calves receiving second dose than that of the first dose at both 6- and 12-month. On the other hand, no significant effect was found among the studied male groups at both ages as showed in **Figure** 1.

3.1.2. Serum level of LDH

The results revealed significant increase in LDM levels in the treated group than the control groups with no sex differences (**Figure 2**).

3.2. Hematological analysis

The serum level of both erythrocytes and leukocytes parameters were compared among different groups as well as among the first and second injection of the same group.

3.2.1. Analysis of leukocyte parameters

3.2.1.1 Analysis of WBCs count

Our results show a significant decrease in WBCs count in the treated group than that of the control groups. Furthermore, sex difference was observed in the values of WBCs, whereas a significant decrease in the WBCs count was observed in the female treated calves receiving second dose than that receiving first dose at the group of 12-month age (**Figure 3**).

3.2.1.2 Analysis of lymphocyte count

Our results show no significant difference in lymphocyte count among the treated and control groups as well as between the second injection and first injection of the same group in all treated calves (**Figure 4**).

3.2.1.3 Analysis of MID

No significant difference could be observed among the treated and control groups as well as between the second injection and first injection of the same group in all treated calves (**Figure 5**).

3.2.1.4 Analysis of Granulocyte count

Significant difference was observed in the granulocytes parameters in the male treated group receiving second dose than that receiving first dose while there was no Significant difference between control and treated femal in firest and second injection (**Figure 6**).

3.2.2. Erythrocyte parameters

3.2.2.1. Analysis of the HGB concentration

Our results showed no significant difference among groups receiving first and second injected doses as well as among treated and control groups (**Figure 7**).

3.2.2.2. Analysis of the HCT parameters

The current study revealed a significant decrease in HCT in the treated group than the control groups (**Figure 8**).

3.2.2.3. Analysis of the Erythrocyte count

The present investigation revealed significant decrease in RBCs in the treated group than the control groups (**Figure 9**).

3.2.2.4. Analysis of the MCV parameters

The results showed no significant differences between all treated groups in MCV levels (**Figure 10**).

3.2.3. Analysis of platelet count

The data celebrated no significant difference among groups receiving first and second injected doses as well as among treated and control groups (**Figure 11**).

4. Discussion

Tilmicosin is a broad-spectrum antibiotic that has been suc-cessfully used to treat respiratory illnesses in sheep, cattle, pigs, and rodents after being administered subcutaneously. (Naccari et

al., 2001; Xiong et al., 2019; Zhang, Zhao, Liu, Liu, & Li, 2017). However, acute cardiac toxicity has been observed in increased dose or following intravenous injection of 10 mg tilmicosin/kg b wt (Er, Tras, & Cetin, 2014; Oda & Derbalah, 2018). Interestingly, it has been reported that the effect of tilmicosin as long-acting antibiotic extended for three day following single injection (Hoflack, Maes, Mateusen, Verdonck, & de Kruif, 2001; A. E. Ibrahim & M. M. J. C. J. Abdel-Daim, 2015; Ortega, Alfonseca-Silva, Posadas, Tapia, & Sumano, 2020). The present study was performed to investigate the influence of increasing the period of tilmicosin action via administering two dose as prophylactic trial and the effect of two doses on the blood pa-rameter and cardiac function were compared among the studied groups as well as among the two doses in the same group.

LDH and CK-MB have been described as indicators of damaged cardiac myocytes (Bodor, 2016; Zhu et al., 2022). The present investigation revealed significant increase in CK-MB level after second injection in the treated female calves' groups at both 6- and 12-month age when second injection compared to first one, but no significant effect on male treated calves in both ages. Furthermore, a significant increase was observed in all treated groups after second injection in comparison to the control group. However, no significant effect was found among both treated and control group after first injection. This suggest sexual difference for the effect of second dose of tilmicosin whereas more side effect could be ob-served in the female than the male treated groups. Like this, it has been documented that tilmicosin administration in many species can cause cardiac toxicity and an increase in CK-MB levels (A. E. Ibrahim & M. M. Abdel-Daim, 2015; Kart, Yapar, Karapehlivan, & Citil, 2007; Said, El-Nabtity, Selim, & Fadel, 2016).

On the other hand, for the LDH level, no sex difference was observed among the treated groups after the second dose whereas our results revealed increase in LDH level in both male and female calves after the second dose than that of first dose. Furthermore, a significant increase in their level was observed in all treated groups after second injection in comparison to that of control group. However, significant increase was observed in the LDH level only in the female treated groups at both 6 and 12 months after first dose in comparison to that in the control group with no significant effect after first injection in the male treated calves at both 6- and 12-months age when compared with that of the control groups. We propose that this disparity between CK-MB and LDH levels results from the existence of LDH in many organs rather than the unique localization of CK-MB in the heart alone (Talwar, Kondareddy, & A, 2017).

For hematological analysis among different studied groups and doses, our results revealed significant decrease in WBCs count after first and second injection on treated calves when compared to that of the control one. Furthermore, at 12 months, the female treated group but not the male showed significant decrease in leukocyte count after second injection on female 12-month calves when compared with first injection. Similarly, previous reports revealed decreasing effect of tilmicosin on WBCs count in chickens, rodents, and piglets (Altunok et al., 2002; Elazab, Gomaa, & Sobhy, 2014; Nerland et al., 2005; Said et al., 2016).

When comparing the erythrocyte parameters between the treatment group and the control group, we found a substantial reduction in HCT after the second injection but not after the first. In mice, this decline has already been shown (Gheith et al., 2015). In terms of RBC count, we found a substantial drop in treated females at 6 months and treated males at 12 months when compared to the first injection, but no significant differences were seen in the other groups. This concurs with other earlier reports (Altunok et al., 2002; Bedeer, El-Diasty, & M.G, 2021; Gheith et al., 2015; Said et al., 2016). Moreover, our data revealed a sig-nificant decrease in RBCs count in all treated groups after first and second injection when compared to that of control groups.

A prior report showed that tilmicosin therapy significantly in-creased granulocyte count. (Buret, 2010). Interestingly, our data revealed only significant difference in the treated male groups at 6 months between second and first injection, with no difference among other treated groups, as well as among the treated and control groups. Furthermore, no significant difference could be observed in lymphocyte count among the treated and control groups as well as between the second injection and first injection of the same group in all treated calves.

In contrast to other parameters including MID count, HGB, MCV, platelet counts, our data

revealed no significant difference among groups receiving first and second injected doses as well as among treated and control groups. This goes in line with other previous reports (Xie et al., 2011).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available within the article text, and figures.

Conflicts of Interest: The authors declare no competing interests.

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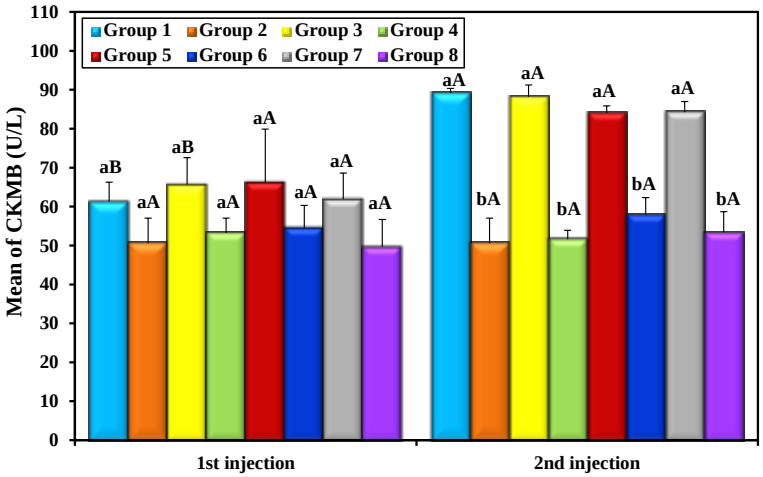

Figure 1. Graph showing the serum level of CK-MB among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

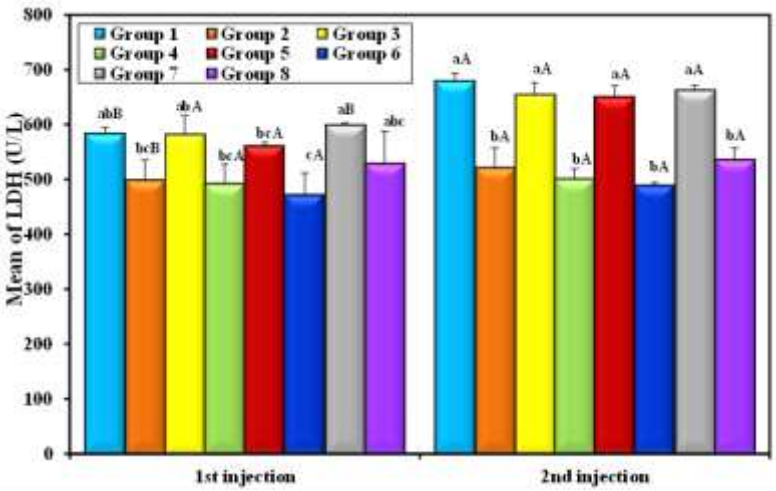

Figure 2. Graph showing the serum level of LDH among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

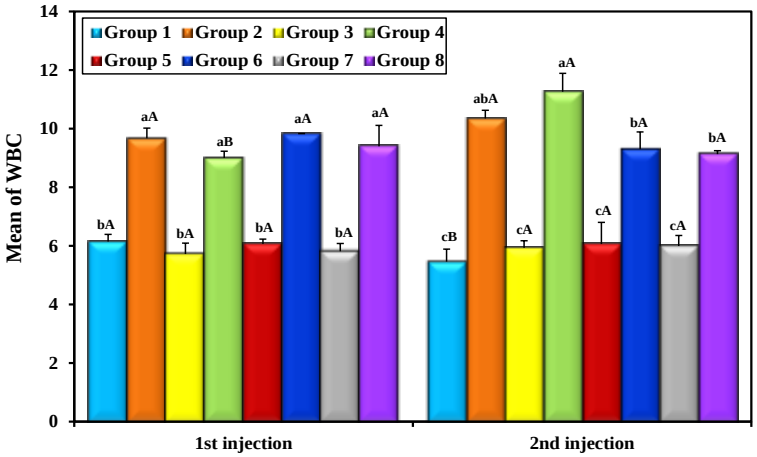

Figure 3. Graph showing the WBCs count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with

small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

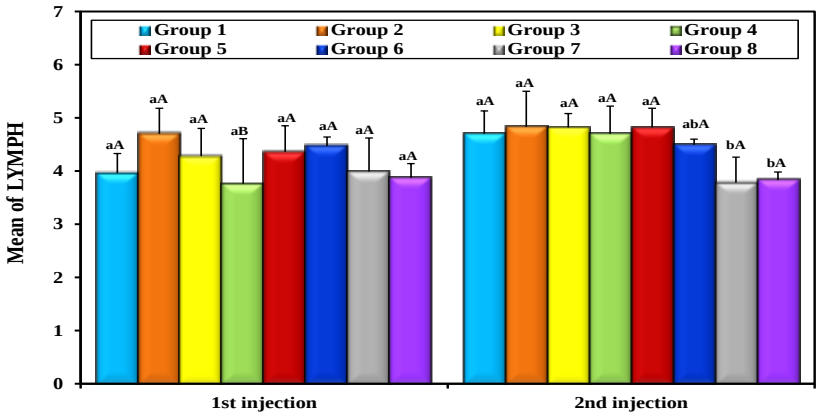

Figure 4. Graph showing the lymphocytes count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

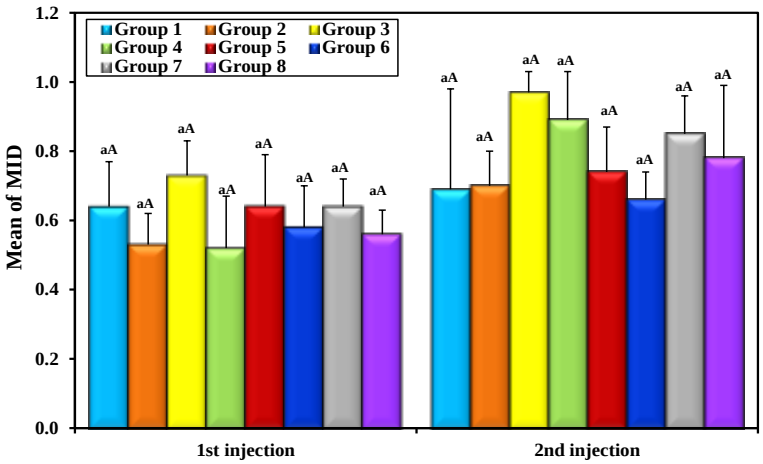

Figure 5. Graph showing the MID count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

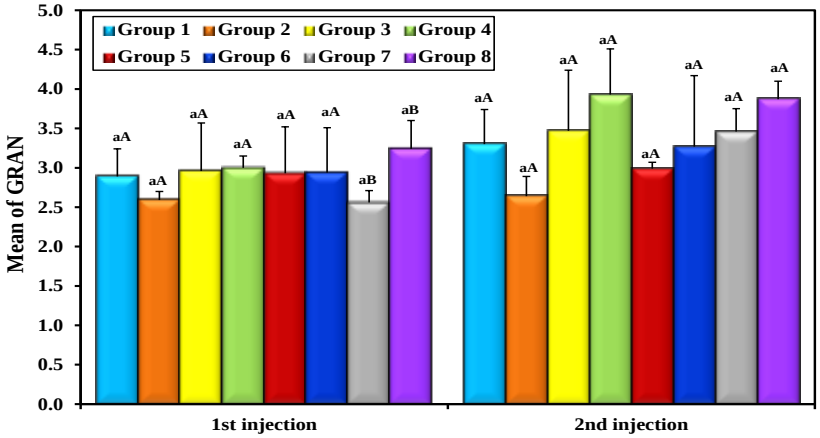

Figure 6. Graph showing the granulocytes count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

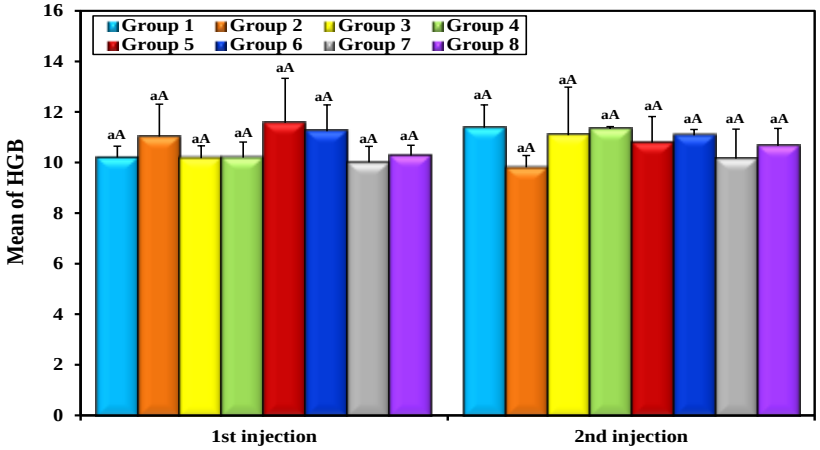

Figure 7. Graph showing the HGB concentration among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e. Means with Different letters are significant).

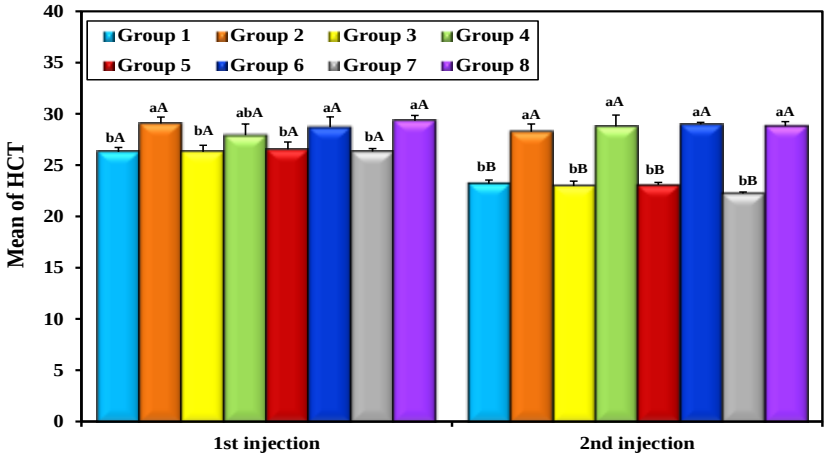

Figure 8. Graph showing the HCT values among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

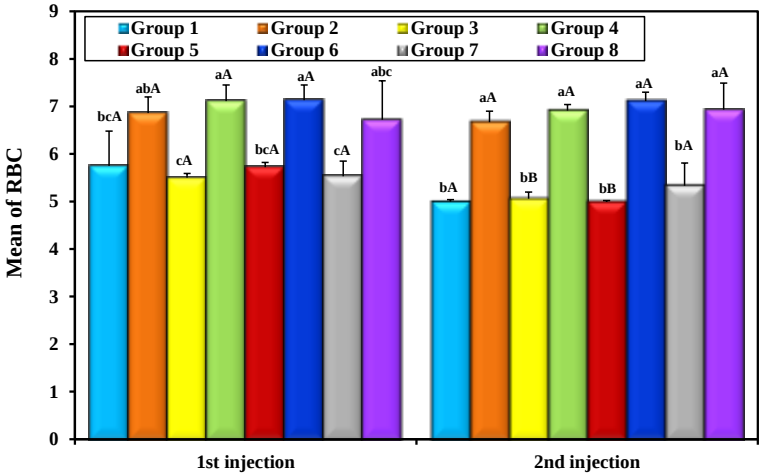

Figure 9. Graph showing the RBCs count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

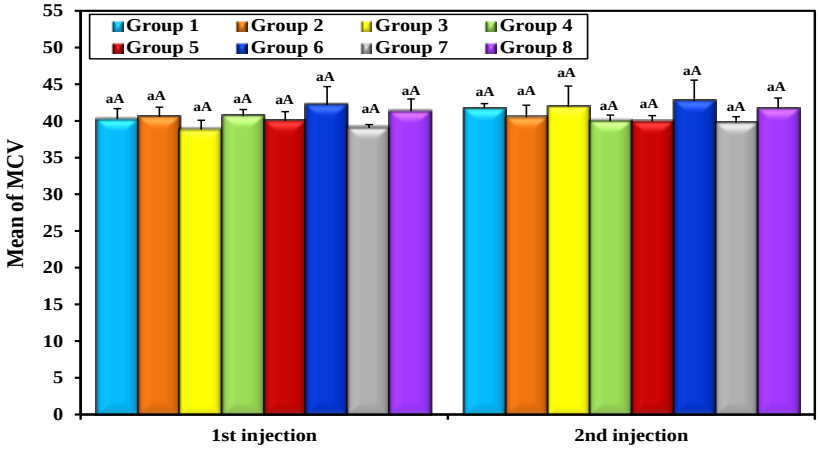

Figure 10. Graph showing the MCV values among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).

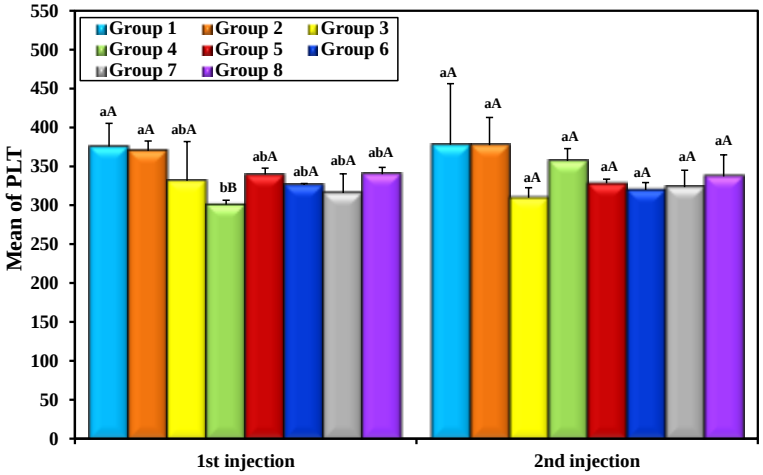

Figure 11. Graph showing the platelet count among the studied groups as well as among the two injections of the same group. Data are expressed as the mean ± SD and were analyzed by ANOVA with repeated measures followed by Tukey's post hoc test. Means with small common letters are not significant between different groups and Means with Capital common letters are not significant between 1st injection and 2nd injection (i.e., Means with Different letters are significant).