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Sub-chronic drug toxicity investigation of highly active anti-retroviral therapy (HAART) in Wistar rats

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Abstract

The widespread use of highly active antiretroviral therapies (HAART) for HIV/AIDS treatment has significantly improved patient outcomes. However, long-term administration of these therapies can have unintended consequences. This study investigated the potential adverse effects of LZN, a combination antiretroviral drug, on hematological parameters and serum biomarkers in wistar rats. A total of forty-eight (48) female Wistar rats were divided into four groups: a control group and three groups administered LZN orally at doses of 25, 50, and 100 mg/kg for 90 days. Hematological parameters and serum biomarkers were assessed at 30, 60, and 90 days. Result obtained in this study revealed that the LD_{50} of the LZN drug sample was calculated to be 2154.07mg/kg body weight in rat model which is an indication that the drug sample is of low toxicity or is relatively safe with moderate safe margin. The haematological result showed that prolonged administration of LZN had an adverse effect on the Hb, PCV, RBC, TWBC, platelets count and the MCH indices after 60 days of administration, with a resultant microcytic hypochromic form of anaemia and classic neutropenia (dose and time dependently). The significant increases in the serum levels of ALT, AST, ALP, creatinine and urea (the liver and kidney biomarkers) in the rats administered the graded doses of the LZN drug sample at prolonged administration, suggested hepatocellular damage and nephrotoxicity, with significant alteration of electrolytes metabolism of sodium, chloride, potassium and bicarbonate concentrations (disturbance in acid-base balance). Also, the abnormal milieu of GSH and MDA serum levels evidently indicate an increased oxidative stress and lipid peroxidation caused by prolonged administration. LZN, while effective for HIV/AIDS treatment, can have long-term adverse effects on hematological parameters, organ function, and electrolyte balance. These findings emphasize the importance of regular monitoring and potential adjustments in therapy to mitigate these risks. Further research is needed to fully understand LZN's impact and inform optimal treatment strategies.

Keywords: Antiretroviral therapy; Drug toxicity; LZN; Organ damage; Hepatotoxicity; Oxidative stress

1. Introduction

Highly active antiretroviral therapy (HAART) is widely regarded as the most effective medical approach for managing and treating patients infected with human immunodeficiency virus type 1 (HIV-1), significantly extending their life expectancy [1, 2, 3]. This therapy relies on the combined administration of two or more antiretroviral drugs, typically including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) alongside protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs). Since the introduction of HAART, there has been a substantial improvement in the overall prognosis for HIV infection, marked by effective viral load suppression, enhanced immune function, and reduced risk of opportunistic infections. This has led to a notable decline in AIDS-related morbidity and mortality among individuals with HIV-1 [4, 5, 6].

The efficacy of highly active antiretroviral therapy (HAART) is undeniably significant, yet its associated toxicity remains a critical limiting factor, often resulting in therapy discontinuation, regimen changes, and non-adherence to therapy [7,

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8]. Side effects of HAART include liver toxicity, hematuria, decreased bone density, cardiovascular disease, gastrointestinal tract infections, hypersensitivity reactions, lactic acidosis, lipodystrophy, myopathy, hepatotoxicity, and renal impairment [9, 10, 11, 12, 13]. The combination of drugs used in HAART contributes to metabolic changes, leading to conditions such as lipodystrophy syndrome and lactic acidosis [14]. The effects of HAART are diverse and result from a complex interaction between the virus, the drugs, and host factors. While the development of new antiretroviral agents is ongoing, continuous efforts are necessary to optimize the effectiveness of current treatments, including better understanding and management of adverse effects.

2. Material and methods

2.1. Study Location

The study was carried out in the Research and Teaching Laboratory Unit; Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike (MOUAU). The study site is located in the South Eastern Zone of Nigeria, at the latitude of 5°29'N and Longitude 7°32'E and altitude of 123m above sea level with an annual rainfall of 2177m, average ambient temperature of 22.32 °C and relative humidity of above 50-90 (National Root Crops Research Institute-NRCRI, 2017).

2.2. Equipment

Some of the equipment used includes: Mettler (analytical) balance, Beakers, Dissecting kit, Analytical weighing balance, Gastric tube (gavage), Pasteur pipettes, Haemocytometer, Haemoglobinometer, Adams microheamatocrit reader, Haematocrit centrifuge, etc.

Drug used: LZN which is a highly active anti-retroviral therapy (HAART).

2.3. Acute Toxicity Testing

This was done using Lorke method [15]. A total of 28 adult mice were used for the study. The study was carried out in two phases. In the first phase, four groups; A-D of 4 mice in each group were given (orally) distilled water (5ml/kg), 10 mg/kg, 100 mg/kg and 1000mg/kg of the LZN drug, respectively at 65 mg/ml concentration (one tabulate containing 650 mg was dissolved in 10 ml of distilled water). Mice in group A served as the normal control. In the second phase, four groups; E-G with 4 mice in each group, were given (orally), 1600 mg/kg, 2900 mg/kg and 5000mg/kg of the LZN drug respectively. The mice were observed for signs and symptoms of (acute) toxicity and mortality over a period of 3, 6, 12, 24, 36 and 72 hours, and were allowed for another 14 days to observe any delayed toxicity.

The median lethal dose (LD_{50}) of the drug sample was then calculated using the formula adopted by [16].

$LD_{50} = \sqrt{}$ (least dose with mortality × Highest dose without mortality)

Table 1 Acute toxicity testing

Group	Dose of Drug	Number of mice	Number of dead mice	% Mortality
A	5ml/kg D/H_20	4 mice		
B	10 mg/kg	4 mice		
C	100 mg/kg	4 mice		
D	1000 mg/kg	4 mice		
E	1600 mg/kg	4 mice		
F	2900 mg/kg	4 mice		
G	5000 mg/kg	4 mice		

2.4. Chronic Toxicity Study

The chronic toxicity study lasted for 90 consecutive days.

2.4.1. Preparation of graded doses and the concentration of LZN combination therapy

For longer administration the graded doses; 25, 50 and 100 mg/kg of the LZN drug were prepared/calculated using lower concentration of 10.83mg/ml (one tablet containing 650 mg dissolved in 60 ml of distilled water).

2.4.2. Ethics approval, animal treatment and experimental design

Forty-eight pathogen free adult female wistar rats (aged 10-11 weeks old) purchased from the Animal Laboratory House, Department of Physiology and Pharmacology were used for this study. All procedures regarding the handling of the animals were carried out in strict compliance to the institutional ethical instructions for the work, as well as adequate consultations to the EEC guidelines to laboratory animal care and use [17]. The study protocol was approved by the Ethical Committee, College of Veterinary Medicine, MOUAU. All the rats were housed in stainless steel cage, given fresh water and were fed standard pellets (Vital Feed®) *ad libitum*. and maintained under ambient temperature and 12h light/dark conditions, de-wormed with mild broad spectrum anthelminthic four weeks before the commencement of the study. The animals were randomly distributed into four treatment groups (1, 2, 3 and 4) and treated as indicated below;

- Group 1 served as the control administered distilled water.
- Group 2: received 25mg/kg LZN drug
- Group 3: received 50mg/kg LZN drug
- Group 4: received 100mg/kg LZN drug

2.5. Laboratory investigations

2.5.1. Blood collection and sampling

The experimental rats were properly restrained for the collection of blood via median canthus. Using the heparinized capillary tubes, 2ml of the blood were collected into separate bottles containing ethylamine tetra-acetic acid (EDTA) for haematological studies, and four milliliters (4 mls) into plain sterile test tubes with screw caps for serum biochemical studies according to the procedure of [18, 19]. The plain test tubes were kept slanted on a bench at room temperature for about 2hrs to allow the blood to clot and yield serum. The clotted blood samples were centrifuged at 3500 revolution per minute (rpm) for 10 minutes and the sera obtained were separated into clean labeled tubes and stored at -20^{o}C until needed for analysis. Meanwhile, the blood collected in EDTA coated test tubes were used immediately for haematological evaluations.

2.5.2. Determination of haematological parameter and indices

Total erythrocyte count (Red Blood Cells)

The Total Erythrocyte count was evaluated using haemocytometer as described by [20]. To 3.98 mL of diluting fluid, 0.02 mL of blood was added, and the mixture was carefully used to charge the improved Neubauer counting chamber. The cells were allowed to settle in a moist chamber for 3-5min. The ruled area of the counting chamber was first focused at × 10 objective of the microscope prior to the counting of the total red blood cells at × 40 objective.

Packed cell volume

The packed cell volume (PCV) was estimated using microhaematocrit centrifuge and tubes. The centrifuge was operated at 3000 rpm for 10 minutes and PCV which is the zone below the Buffy coat was read using a micro-capillary reader [20].

Haemoglobin concentration (Hb)

The haemoglobin concentration was obtained using modified cyanmethaemoglobin method of [21]. 0.02 ml of blood was deposited into a clean cuvette and then diluted with 5 ml of cyanmethaemoglobin reagent (modified Drabkin fluid). After the addition of the diluents, the cuvette was stoppered and inverted 2-3 times and allowed to stand for 10 min for maximum conversion of Hb to cyanmethaemoglobin. The absorbance of the resulting mixture was read using spectrophotometer (Beckham Coulter, Model B U520, Austria) at wavelength of 540 nm against the blank. The optical density at 540 nm was recorded and compared with the reading, obtained using a standard solution of cyanmethaemoglobin.

Total/differential white blood cell count

The Total and differential white blood cell counts were obtained by peroxidase cytochemical reaction [20, 22, 23]. In the peroxidase cytochemical reaction, erythrocytes were lysed and 4 - chloro - 1 – naphthol reagent serves as a substrate which enables hydrogen peroxide to form a dark precipitate at the sites of peroxidase activity in the granules of some leucocytes. Neutrophils, eosinophils, and monocytes were stained based on their levels of peroxidase activity. The lymphocytes, basophils and large cells contain no peroxidase and hence remain unstained.

Determination of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from Hb, PCV and red blood cell counts as described by [21] using the formula:

$$\text{MCV } (\mu m^3) = \frac{\text{PCV} \times 10}{\text{Number of erythrocytes per } \mu\text{L blood} \times 10^{-6}}$$

$$\text{MCH } (\mu g) = \frac{\text{Haemoglobin (g/dL)} \times 10}{\text{Number of erythrocytes per } \mu\text{L blood} \times 10^{-6}}$$

$$\text{MCHC (g/dL) or g\%} = \frac{\text{Haemoglobin (g/dL)} \times 100}{\text{PCV (mL / dL)}}$$

2.5.3. Determination of serum biochemical parameter and indices

Serum Aspartate Aminotransferase (AST) and Serum Alanine Aminotransferase (ALT)

Serum AST and ALT activities was determined using the standard colorimetric method of Randox Glutamic-oxaloacetic transaminase test kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY). The procedure was carried out according to the manufacture's prescription.

Serum Alkaline Phosphatase (ALP)

Determination of serum ALP activity was done using the phenolphthalein monophosphate method for the in vitro determination of ALP in serum using Quimica Clinica test kit (QCA, CN-340km 1081- P.O BOX 20-E43870 AMPOSTA/Spain). The procedure was carried out according to the manufacture's prescription.

Determination of Serum Urea and Creatinine concentration

These were done using the method of [24, 25] respectively.

$$\text{Urea (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{concentration of standard}}{1}$$

$$\frac{\text{Change in absorbance of sample}}{\text{Change in absorbance of standard}} \text{ x } 2 = \text{creatinine (mg/dl)}$$

2.5.4. Determination of Malondialdehyde (MDA) Concentration

MDA concentration was determined by the colorimetric method of [26]. To 0.1 ml of sample in test tube was added 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (0.05 M sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15min. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10min. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of plasma.

The concentration of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$).

$$MDA\ (nmol/ml) = (OD \times 1000000)/ E_{532}$$

Where:

- E_{532} = Molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$)
- 1000000 = conversion of mMol to nMol

2.5.5. Determination of reduced glutathione (GSH)

Reduced glutathione was determined by the method of [27]. 0.2 ml of sample was mixed with 1.8 ml of EDTA solution. To this 3.0 ml of precipitating reagent was added, mixed thoroughly and kept for 5 minutes before centrifugation. To 2 ml of the filtrate, 4 ml of 0.3 M disodium hydrogen phosphate solution and 1 ml of DTNB reagent were added and the colour developed was read at 412 nm in spectrophotometer. Standard solutions containing 10 and 20 mg/dL of reduced glutathione were treated similarly. The values were expressed as mg/dl for plasma

$$Conc\ of\ GSH\ (umol/l) = OD_{test}/OD_{std}\ X\ standard\ concentration.$$

2.5.6. Statistical analysis

Descriptive and inferential statistics were used to analyze the data obtained from this study. Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Statistics, UK). The analyzed results were expressed in terms of means ± standard errors, presented in tables, bar chart and line graphs where appropriate. One-way analysis of variance (ANOVA) coupled with appropriate post-hoc statistics were conducted to compare the days of sample collection and to separate means of the effect of prolonged administration of LZN drug on blood profile and organ weights at the graded doses, while statistical confidence was set at 95 % ($p < 0.05$).

3. Results

3.1. Acute toxicity

The acute toxicity result showed that all the mice that received the highest (5000 mg/kg) dose of the drug sample showed obvious signs of weakness, with an increased beating of the heart after 3hrs post administration of the drug sample, while those that received 2900 mg/kg showed mild to moderate weakness, 6hrs post administration, whereas, the mice in other groups did not show noticeable signs of weakness or toxicity. Twenty-four hours post administration, 3 out of 4 (75%) mortality was recorded in 5000 mg/kg group, while 2 out of 4 (50%) mortality was recorded in the 2900 mg/kg group, and no mortality in other groups. The mice were allowed for 14 days during which their vital signs were monitored daily for any delayed toxicity signs. There was no other death or toxicity signs observed during the 14-day window.

3.2. Effect of Long-Term Use of Lamivudine, Zidovudine and Nevirapine on Liver Enzyme Activities of Wistar Rats

The result of the effect of LZN on the liver enzyme activities showed significant alterations in the mean value of ALT, AST, and ALP activities across the duration and treatment groups as shown in figure 1. The serum ALT and AST activities were significantly elevated at days 30 and 90 following administration as the doses were increased (dose dependent manner) while after 60 days, the serum ALT and AST activities of the group administered 25 mg/kg was significantly higher (hepatotoxicity) than those of 50 mg/kg and 100 mg/kg groups which were within the normal level as that of the control. ALP activity at day 30 were significantly increased in the treated groups as the doses were increased and 60 days after administration, the ALP serum level returned to a mean level comparable with that of the control group, but after 90 days, the level in the 50 mg/kg was significantly increased compared with other treated groups and that of the control group.

3.3. Effect of Long-Term Use of Lamivudine, Zidovudine and Nevirapine on Kidney Function Biomarkers of Wistar Rats

The serum creatinine levels remained unchanged at 30 and 60 days of administration, but significantly increased at 90 days across all dose levels compared to the control group. Urea concentration was significantly increased at 30 and 90

days in a dose-dependent manner, though it decreased after 60 days in the 50 mg/kg and 100 mg/kg groups. Serum electrolyte levels varied significantly with prolonged LZN administration. At 30 days, sodium levels were significantly higher in the 50 mg/kg group compared to the 25 mg/kg and control groups. By 60 days, sodium levels increased in the 25 mg/kg group but decreased in all LZN-treated groups compared to controls at 90 days. Chloride levels remained stable at 30 days but decreased significantly at 60 and 90 days with higher doses (50 and 100 mg/kg). Potassium levels were significantly elevated (hyperkalemia) in the 100 mg/kg group at 30 days but decreased in the 25 mg/kg group at 60 days, and further decreased in the 25 and 50 mg/kg groups at 90 days. HCO3 levels also showed significant variations among the treated groups and across the treatment duration.

3.4. Effect of Long-term Use of Lamivudine, Zidovudine and Nevirapine on Haematology Parameters (Full Blood Count) of Wistar Rats

The results of the long-term administration of the combination drug Lamivudine, Zidovudine, and Nevirapine (LZN) on haematological parameters showed varied effects over time. Hemoglobin (Hb) levels remained unchanged after 30 days of administration, but after 60 days, there was a significant reduction. However, by the 90th day, Hb levels returned to values comparable to the control group. Similarly, the Packed Cell Volume (PCV) was not altered after 30 days, but a significant decrease was observed after 60 days, followed by a return to mean level comparable to control groups by day 90, as illustrated in Fig 3.

The Red Blood Cell (RBC) count also showed no changes in the first 30 days, but after 60 days, the count significantly increased with higher doses. By the 90th day, RBC counts stabilized within a range comparable to the control group. The Total White Blood Cell (TWBC) count, as presented in Fig 4.13, significantly decreased at day 60 with increased doses compared to the control value. On days 30 and 90, TWBC mean values were not significantly different from the control group.

Platelet counts, as shown in Fig 4, remained consistent throughout the treatment period (30, 60, and 90 days), with no significant alterations compared to the control values. However, in the low dose group (25 mg/kg), the mean platelet count was significantly lower than in the medium dose group.

The erythrocyte indices, including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC), showed similar patterns. Within 30 days of LZN administration, these indices were not significantly different from the control group. The MCV and MCH values significantly decreased with increased doses up to 100 mg/kg after 60 days of administration. The MCHC at 60 and 90 days also significantly decreased in the low and medium dose groups compared to the control group.

Lymphocyte counts showed a significant increase and decrease at low and medium doses, respectively, after 60 days, while counts in the high-dose group remained within normal mean levels, suggesting that LZN administration may induce transient lymphocytosis after 60 days. Neutrophil counts exhibited a similar pattern, with significant increases and decreases at low and medium doses after 60 days, indicating a transient neutropenia. Monocyte and eosinophil counts remained consistent with the control values throughout the study period.

3.5. Effect of Longterm Use of Lamivudine, Zidovudine and Nevirapine on GSH and Thiobarbituric Acid Reactive Substance (TBARS) Assay

The results of the antioxidant effects of the LZN drug, as presented in Fig 6, showed significant changes in Glutathione (GSH) levels. The mean GSH levels were significantly increased in rats administered with 25 mg/kg and 50 mg/kg doses after 30 and 60 days, respectively, compared to the control group. However, rats administered 100 mg/kg showed GSH levels comparable to the control. After prolonged administration (90 days), there were no significant differences in GSH levels across all treated groups compared to the control. Similarly, the antioxidant effects of the LZN drug on Malondialdehyde (MDA) levels, as shown in Fig 6, revealed significant alterations. The mean MDA levels were significantly increased in all LZN-treated groups after 60 days of treatment. No significant changes were observed in the MDA levels at 30 and 90 days compared to the control group.

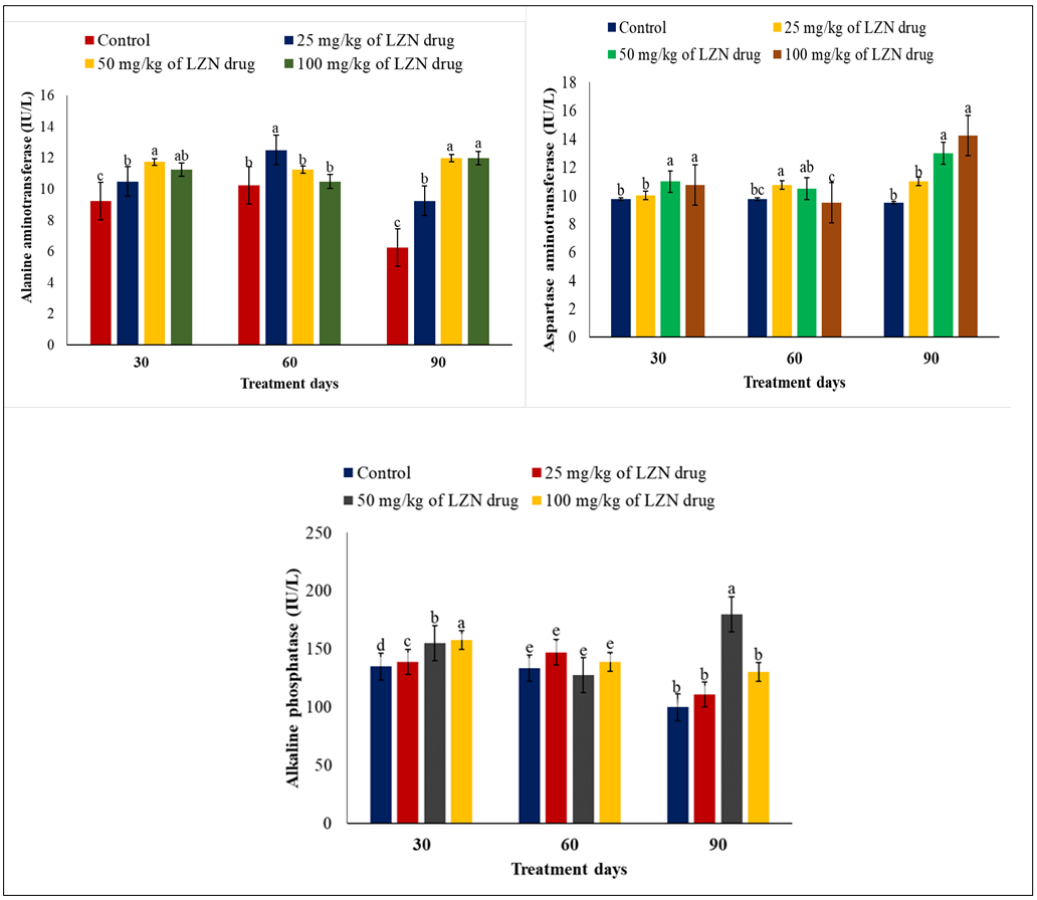

Figure 1 Effect of prolonged administration of LZN on ALT, AST and ALP activity

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences. ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase.

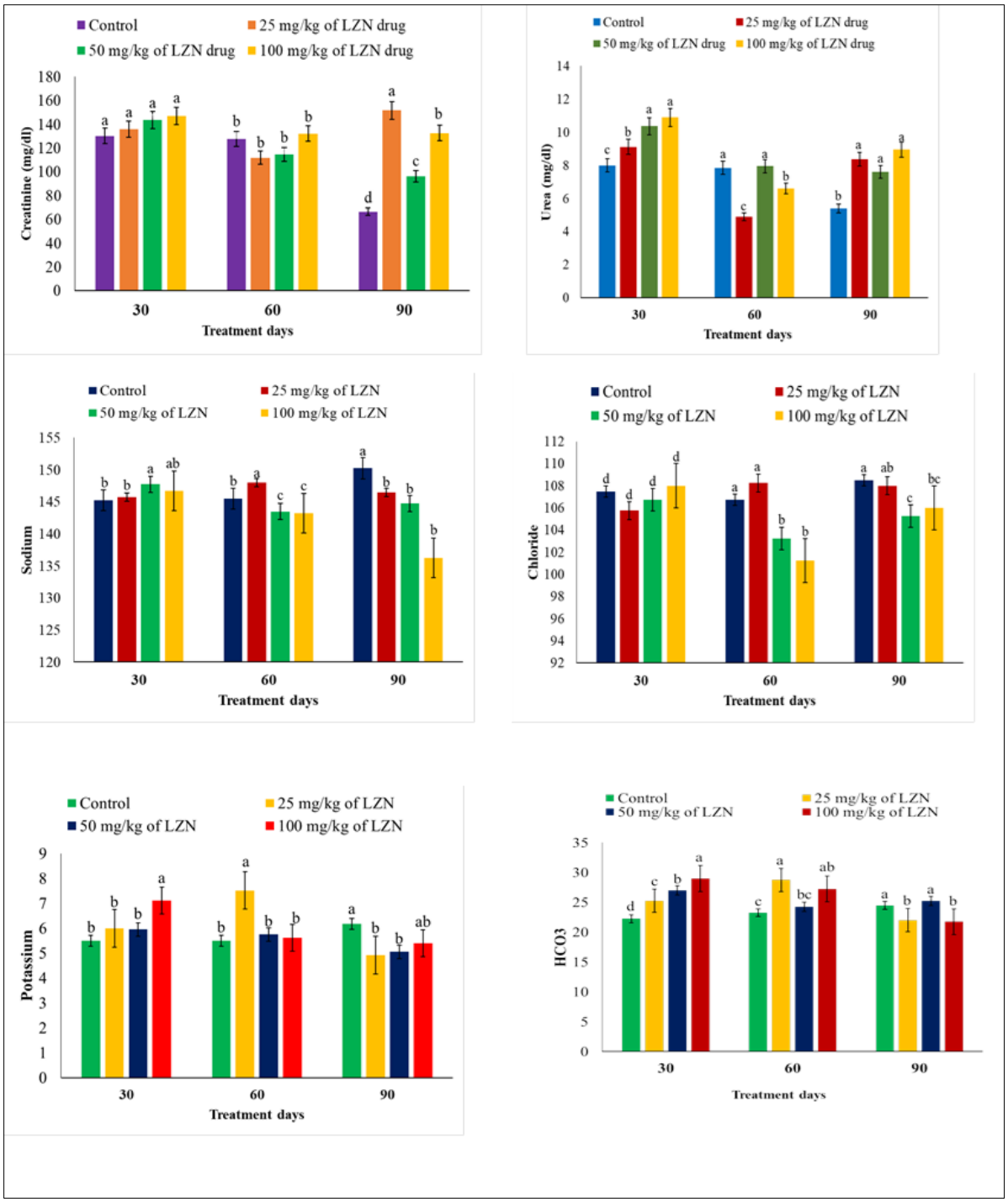

Figure 2 Effect of prolonged administration of LZN on kidney function biomarkers

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences.

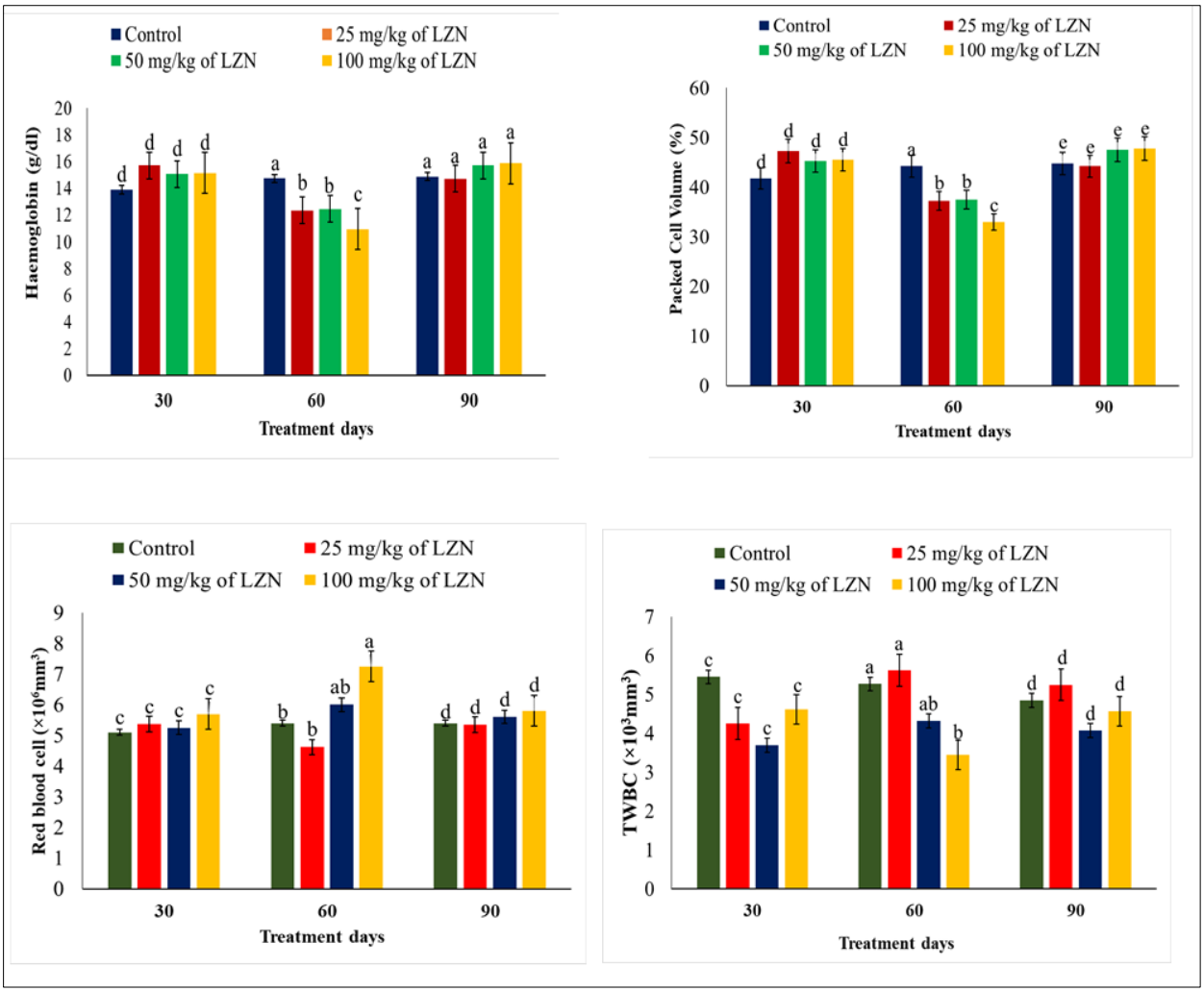

Figure 3 Effect of prolonged administration of LZN on haemoglobin, packed cell volume, red blood cell and total white blood cell (TWBC)

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences.

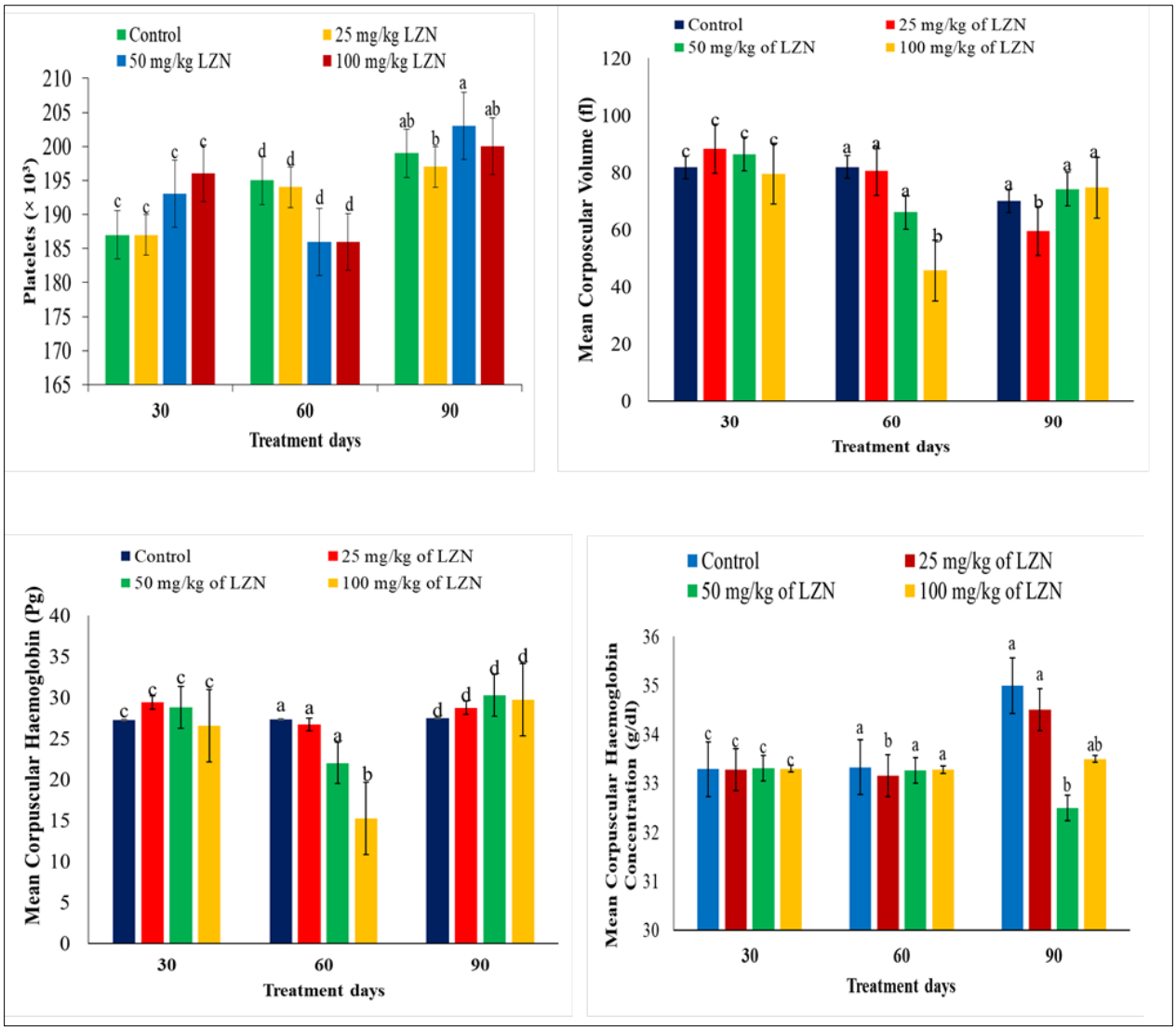

Figure 4 Effect of prolonged administration of LZN on platelets, mean corpuscular volume, mean corpuscular haemoglobin (pg) and mean corpuscular haemoglobin

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences.

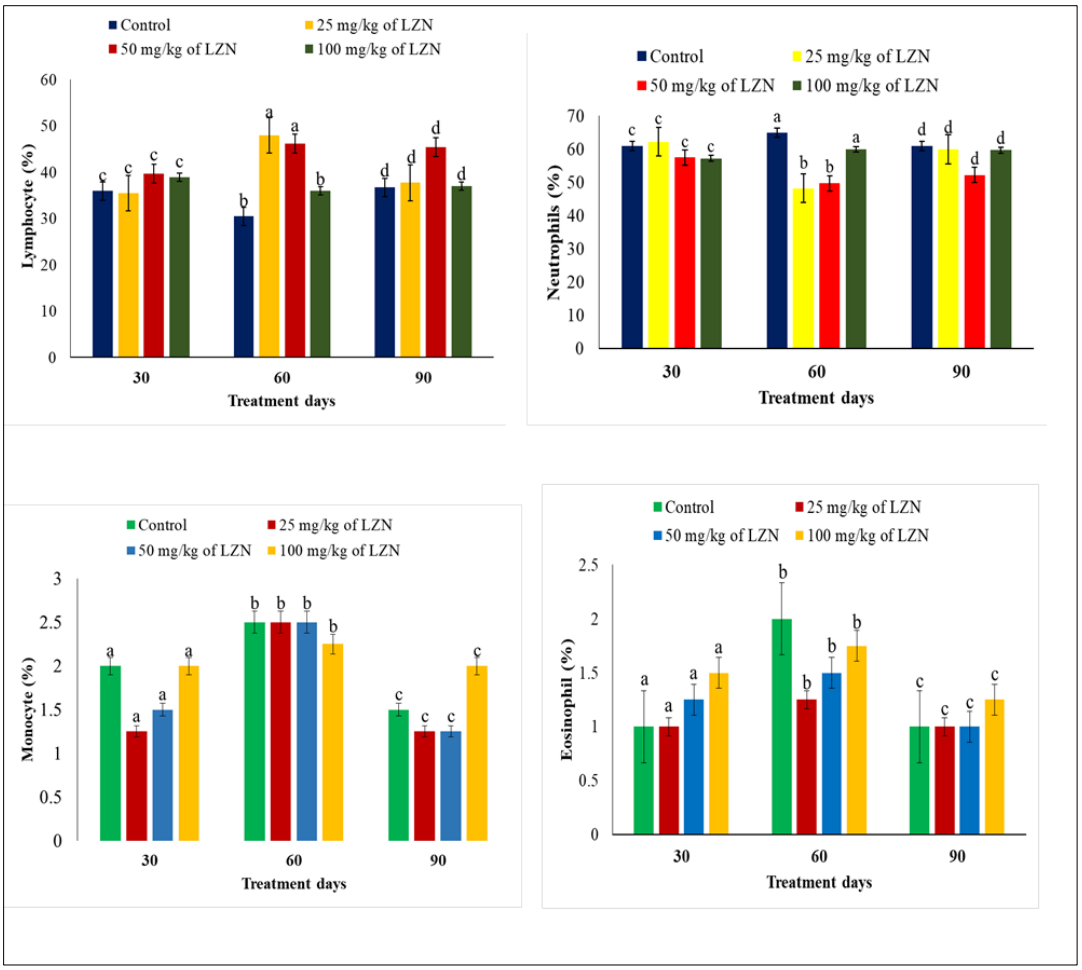

Figure 5 Effect of prolonged administration of LZN on lymphocyte, neutrophils, monocyte and eosinophil.

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences.

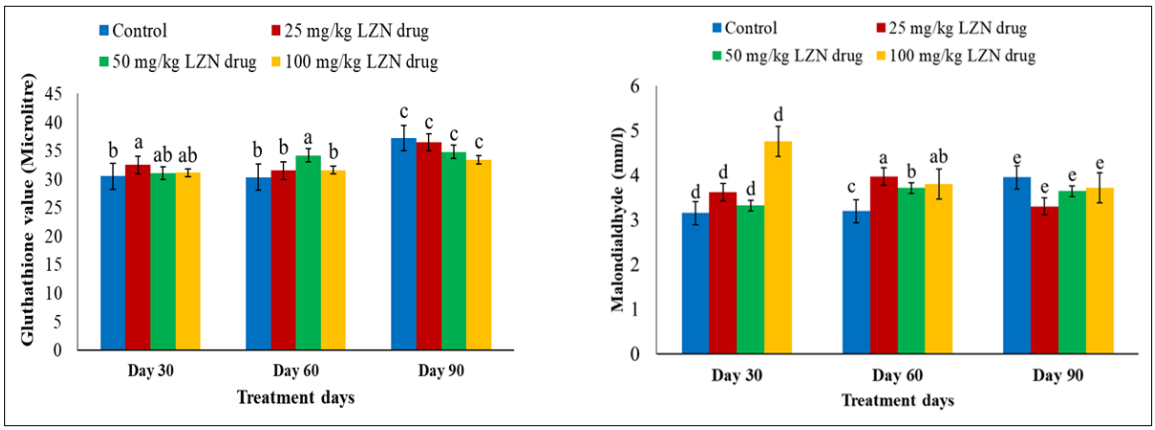

Figure 6 Effect of prolonged administration of LZN on glutathione and thiobarbituric acid reactive substance (TBARS) assay

Values are presented as Mean ± SEM (Standard Error of Mean). Different superscript letters across treatment groups show significant ($p<0.05$) differences.

4. Discussion

Many synthetic drugs and natural plant extracts used in conventional and traditional medicine are often considered safe, despite limited toxicological data or scientific validation [29]. The introduction of the LZN synthetic drug significantly improved HIV management by reducing viral loads, enhancing immune function, and lowering the incidence of opportunistic infections, thereby increasing patient survival rates [5, 6]. However, despite its benefits, clinical studies side effects and toxicities, necessitating acute and chronic toxicological studies using rodent models. Lamivudine, Nevirapine, and Zidovudine are combined into a synthetic nucleoside analogue tablet, with each film-coated tablet containing 150 mg of Lamivudine, 200 mg of Nevirapine, and 300 mg of Zidovudine. Inactive ingredients include colloidal silicon dioxide, hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate, titanium dioxide, and triacetin [28].

The Organization for Economic Cooperation and Development (OECD) classifies synthetic substances with an LD50 value of < 50 mg/kg as very toxic; > 50 ≤ 500 mg/kg as harmful; > 500 ≤ 2000 mg/kg as not harmful or toxic; and ≥ 5000 mg/kg as very safe [30]. Additionally, substances with an LD50 > 1000 mg/kg are generally considered low in toxicity or relatively safe [31, 32]. The calculated LD50 of the LZN drug sample in mice was 2154.07 mg/kg, indicating that it is of low toxicity or relatively safe. The absence of overt toxicity signs or deaths within 14 days post-oral administration across various dose groups (10–1600 mg/kg) further supports the drug's relative safety and moderate safety margin.

Acute toxicity signs in mice with Lamivudine therapy at a dose of 2000 mg/kg, including skin rash, CNS toxicity, metabolic abnormalities, liver toxicity, and diarrhea was reported by [33]. Similarly, Gallant et al. [34] described acute toxicity of Zidovudine in rats at a dose of 4000 mg/kg, presenting with anemia/neutropenia, nausea, fatigue, body pains, rough hair coat, and diarrhea. Studies on Nevirapine's acute effects in rats at 2000 mg/kg showed symptoms such as diarrhea, respiratory distress, weight loss, and elevated rectal temperature [35, 36]. Furthermore, acute cholestatic hepatitis and oxidative stress have also been reported [12, 37].

The low margin of safety observed in the acute toxicity testing is largely attributed to the toxicity levels of the individual components in the combined therapy, as well as adverse interactions among the drug's constituents. This aligns with similar findings of signs of toxicity and mortality in laboratory animals administered Nevirapine, Lamivudine, and Zidovudine [28, 38, 35, 36].

Biochemical parameters such as total protein, albumin, AST, ALT, cholesterol, urea, creatinine, glucose, and triglycerides are critical in monitoring the impact of therapeutic agents at the cellular level [39]. Alanine aminotransferase (ALT), primarily found in the liver and kidney, serves as a marker for liver damage when released due to cell death [40]. Elevated ALP activity can indicate cholestasis and hepatocellular damage [41], while urea and creatinine levels serve as indicators of kidney function and liver health [42, 43]. In this study, significant increases in ALT, AST, ALP, urea, and creatinine in rats administered graded doses of LZN suggest hepatocellular damage and nephrotoxicity. These elevations may result from mechanisms like the generation of toxic species and membrane peroxidation [44, 45, 46].

Electrolytes play a vital role in maintaining cellular functions and acid-base balance. Significant alterations in sodium, chloride, potassium, and bicarbonate levels observed in this study may disturb acid-base balance and blood pressure regulation [47, 48]. Prolonged LZN administration led to metabolic acidosis due to decreased Na+/K+-ATPase activity, aligning with findings that HAART can cause metabolic changes, including lipodystrophy syndrome and lactic acidosis [14].

Hematological analysis showed that prolonged LZN administration adversely affected Hb, PCV, RBC, TWBC, platelet count, and MCH indices, suggesting bone marrow suppression and anemia [34, 35]. Changes in white blood cell counts indicate possible immunosuppression or stimulation depending on the dose, corroborating studies that associate HAART drugs with immune modulation [49].

Oxidative stress parameters indicated elevated levels of malondialdehyde (MDA), suggesting lipid peroxidation and potential cardiovascular damage [50, 51]. An increase in glutathione (GSH) at certain doses may indicate a compensatory response to oxidative stress [52, 53]. These findings suggest that prolonged HAART therapy induces oxidative stress, potentially leading to tissue damage.

5. Conclusion

The combined LZN therapy showed a low to moderate safety margin in Wistar rats. This study revealed that prolonged LZN administration is associated with microcytic hypochromic anemia, electrolyte imbalance, altered protein metabolism, lipid peroxidation, hepatotoxicity, and nephrotoxicity. Therefore, its use should be approached cautiously, particularly for extended therapy. Regular blood profiling and liver and kidney function tests are crucial for monitoring these biomarkers to determine when to adjust or discontinue treatment. Co-administration with blood-building, hepatoprotective, or nephroprotective agents may help mitigate these adverse effects during prolonged therapy.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interest exists in the work.

Statement of ethical approval

All procedures regarding the handling of the animals were carried out in strict compliance to the institutional ethical instructions for the work, as well as adequate consultations to the EEC guidelines to laboratory animal care and use (Louhimies, 2002). Ethical approval was obtained from the Ethical Committee, College of Veterinary Medicine, MOUAU.

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