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Safety evaluation study of lincomycin and spectinomycin hydrochloride intramuscular injection in chickens

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ABSTRACT

This study aimed to investigate the nonclinical safety of lincomycin and spectinomycin hydrochloride (LC-SPH) intramuscular (i.m) doses on target animals (chickens) to provide guidelines for dose level design and side effect monitoring in clinical trials.

A total of 80 healthy Arbor Acres plus broiler chicks were completely randomized and blindly divided into four treatment groups (control, one-time dose, three-time dose, and five-time dose) of 20 chicks each (20 chickens per group). At the age of day 15, all chickens (except the control group) were administered LC-SPH intramuscularly (chest muscles) at different doses of 20 mg/kg.bw, 60 mg/kg.bw, and 100 mg/kg.bw respectively for 9 consecutive days recommended by veterinary international cooperation on harmonization (VICH) guidelines. The chickens had ad libitum access to antibiotic-free feed and water. Feeding chickens were observed twice a day throughout the study. The drug safety was evaluated by complete blood count, biochemical parameters, histopathological, clinical signs, body weight gain, and feed conversion ratio (FCR).

Hence, considering the minor toxicity of 60 mg/kg, our results reveal that intramuscular injection of at least 20 mg/kg body weight has no effects on growth performance, clinical blood parameters, organ coefficient, and histopathological parameters. Thus, a combination of LC-SPH 20 mg/kg body weight i.m injection investigated safe followed daily administration for nine consecutive days in healthy chickens.

It is concluded that the experimental results support the safety of 20 mg/kg body weight in combination for the further clinical research study.

1. Introduction

Poultry is the fastest-growing source of meat and eggs worldwide. This development resulted from proper genetic selection, improved feeding, health management practices, and antibiotics [1]. The Chinese poultry industry is developing from free-range farming to large-scale. The chronic diseases were paid less attention initially, including chronic respiratory disease (CRD), *Staphylococcus aureus,* and *Mycoplasma species infections*. These microorganisms cause high economic losses to the poultry industry because the infections occur in combination with *Escherichia coli* and decrease the performance and feed-to-meat ratio. Tylosin, enrofloxacin, tiamulin, and other drugs may not be very effective when used alone. Therefore, an alternate way to treat the diseases can be the drug combination [2]. The combined application of

Abbreviations: LC-SPH, Lincomycin and spectinomycin hydrochloride; i.m, intramuscular; VICH, veterinary international cooperation on harmonization; FCR, feed conversion ratio; CRD, chronic respiratory disease; GI, gastrointestinal tract; EU, European Union; MRLs, maximum residue limits; GLP, good laboratory practice; CREA, creatinine; GLU, glucose; TP, total protein; UREA, blood urea nitrogen; Ca, calcium; Rpm, revolutions per minute; EDTA, ethylenediamine tetraacetic acid; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, coefficient of variation of red blood cell width; PLT, platelet count; MPV, mean platelet volume; PDW, platelet distribution width; CK, creatine kinase; P, phosphorus; GLO, globulin; A/G, albumin to globulin ratio.

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antibacterial drugs produces synergistic effects in polymicrobial infections. It can expand the antibacterial spectrum, enhance the efficacy, reduce the drug dosage, reduce or avoid drugs toxic effects, reduce or delay the production of drug-resistant strains [3].

Lincomycin isolated from soil bacteria *Streptomyces lincolnensis* var. *lincolnensis* of the order actinomycetes [4,5] binds to the 50S ribosomal subunit and results in protein synthesis inhibition [6]. The bactericidal or bacteriostatic effect depends on the drug concentration at the infection site and infected organism sensitivity [7]. Absorption is very weak through the gastrointestinal (GI) tract, followed orally, and higher through intramuscular administration [8]. The peak plasma concentration reaches 2–4 h and 1–2 h after oral and intramuscular administration, respectively, while liver metabolic transformation is 50 % after oral administration and these metabolites usually retain the action of antimicrobials [8,9]. It treats intestinal and respiratory infections orally, caused by *Mycoplasma* and gram-positive bacteria [10]. Spectinomycin hydrochloride belongs to the aminoglycoside group [11,12], is isolated from *Streptomyces spectalis* [13], and inhibits the synthesis of bacterial proteins [14]. Spectinomycin doesn't undergo significant metabolism. The gastrointestinal tract absorbs a minimal quantity of spectinomycin and is absorbed rapidly after intramuscular administration. In cattle following the i.m route, spectinomycin was completely bioavailable, and the frequent administration does not produce higher tissue concentration as achieved with a single dose [15]. Spectinomycin is strongly bactericidal and concentration-dependent [16] effective against Gram + ve and Gram-ve bacteria [17] and some gram-negative aerobic bacteria, including *Mannheimia haemolytica*, *Pasteurella multocida*, *Escherichia coli,* and facultative anaerobes organisms *Actinomyces bovis* [18]. In combination, both drugs effectively treat piglet diarrhea, *Mycoplasma hyopneumoniae*, and *Mycoplasma pneumoniae* infection, which can cause chronic respiratory diseases in chickens [19]. The ministry of agriculture China has approved the preparation of lincomycin-spectinomycin hydrochloride soluble powder and lincomycin hydrochloride-spectinomycin sulfate soluble powder [2]. The European Union (EU) and China have set maximum residue limits (MRLs) for lincomycin and spectinomycin. In food-derived animals, the maximum residue limits for spectinomycin and lincomycin are 300−5000 μg/kg and 50−1500 μg/kg, respectively. In the United States, MRLs range from 100−4000 μg/kg for spectinomycin in the muscle and liver of chickens and cattle, and 100−600 μg/kg for lincomycin in the liver and muscle of pigs. In animal-derived foods, Japan has established MRLs of 500−5000 μg/kg for spectinomycin and 200−1500 μg/kg for lincomycin [20,21]. However, there is less information on the safety of combined LC-SPH intramuscular injection in poultry.

We conducted this study to investigate the safety of LC-SPH drug via i.m injection (breast muscle) for further clinical applications, dose level understandings, and toxic dose on chickens histopathological, physiological, and hematological parameters to evaluate the adverse reactions and safety of the drug combination.

2. Materials and methods

2.1. Drugs

LC-SPH batch number (20200305) was obtained from Tianjin Ringpu biopharmaceutical co., ltd, China. 15 g of powder consisting of 5 g lincomycin and 10 g spectinomycin was calculated based on the two molecular formulae ($C_{18}H_{34}N_2O_6S$) and ($C_{14}H_{24}N_2O_7$), respectively, and prepared into 100 mL solution. According to the dose rate of 20 mg/kg body weight, a volume of 0.2 mL, 0.6 mL, and 1 mL drug was administered i.m (chest muscles) to one time, three times, and five times dose groups, respectively. The blank control group was injected with normal saline (the volume was the same as the recommended dose group) continuously for nine days recommended by VICH guidelines [22].

2.1.1. Study design and management of birds

The study was approved by Tianjin Bohai agriculture-animal husbandry union institute co., ltd. (NO: IACUC-AQ2002) and performed according to good laboratory practices (GLP) guidelines and Veterinary international cooperation on harmonization for registration of veterinary drugs [22]. A total of 80 healthy Arbor Acres plus chicks were purchased from Yang Li Le (farmer), Xinli Street, Bazhou xinan town, (Langfang City), Hebei province under animals license (No.302158922). Eighty, day-old chicks were confined to a wire cage once received. After receipt, the chicks were prepared for quarantine and kept under the same climatic and hygienic conditions for 15 days. According to the Complete randomized design and blinded study, on day 15th chicks were distributed into four treatment groups of 20 chicks each (20 chickens per group), equally distributing half male and female chickens, weight ranged 0.50 ± 0.20 kg., equally distributing half male and female chickens, weight ranged 0.50 ± 0.20 kg. The chickens had ad libitum access to antibiotic-free feed and water. Feeding chickens were observed twice a day throughout the study. Temperature and humidity were maintained, according to the chicken's age.

2.2. Instruments and reagents

Instruments used during the experiment involve automatic biochemical analyzer *mindray (BS-360S)* and animal blood cell analyzer (BC-2600 Vet), purchased from Shenzhen mindray biomedical electronics co., Ltd. Desktop high-speed refrigerated centrifuge (model H1850R) from Changsha xiangyi centrifuge instrument co., ltd. Electronic analytical balance BSA224S-CW from Sedorius scientific instrument (Beijing) co., ltd. Automatic vacuum dehydrator model HY-TS1090B, paraffin slices baking machine (model HY-HP), and semi-automatic paraffin slicer (model HY3500) from Jinhua huiyou equipment co., ltd. Automatic embedding machine (model TB-FL1) from Wuhan tianzhirui medical technology co., ltd. The histopathological slides were observed with a Nikon eclipse Ci-L light microscope.

The reagents used in the blood chemistry investigation include creatinine (CREA), glucose (GLU), total protein (TP), urea (UREA), calcium (Ca), and inorganic phosphorus detection kit, were purchased from Chongqing Zhong Yuan biotechnology co., ltd China. The hematoxylin dye solution was purchased from Zhuhai beso biotechnology co., ltd China.

2.3. Clinical observation

Clinical signs include breathing, neurological condition (ataxia, paralysis of legs and wings, torticollis), and abnormal defecation, along with adverse reaction, time, degree, and recovery on the injection site observed and recorded during the study.

2.4. Sample collection and blood clinical parameters determination

Two blood samples were collected from the wing vein 24 h after the last administration of all chickens. One sample in ethylene-di-amine-tetra-acetic acid (EDTA) containing tubes for analysis of hematology parameters including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT). The second sample was used to estimate biochemical parameters including, the activities of total protein (TP), creatinine (CREA), blood urea nitrogen (UREA), creatine kinase (CK), calcium (Ca), phosphorus (P), globulin (GLO) and albumin to globulin ratio (A/G). Serum samples were prepared by centrifuging the blood sample at 4000 rpm for 10 min. Such criteria were determined to cover the spectrum of possible toxicity [23].

2.4.1. Organ coefficient

In the end, all chickens were weighed and dissected. The organs

include the heart, liver, spleen, lung, and kidney, were weighted and calculated the ratio of each organ to body weight via the formula, organ index (%) = organ weight/body weight ×100 [23].

2.4.2. Histopathology

The chickens were exsanguinated via jugular vein for lesions observation include swelling, bleeding, and necrosis. The portion of the heart, kidneys, lungs, muscles, and skin tissue was taken and fixed within formalin for post histopathological observation [24].

2.5. Statistical analysis

Test data were analyzed by using statistical analysis software (SPSS Statistics 17.0). One-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test was used to evaluate different means among treatments. The results were expressed as mean ± standard deviation.

3. Results

3.1. Clinical observation

No mortality and morbidity were observed during the study. The chickens in each group exhibited normal behavior, drinking water, exercise, and no neurological signs were observed (ataxia, paralysis of legs and wings, torticollis). The injection site completely recovered after two weeks of administration. All the chickens appeared normal without any significant clinical signs of intoxication during the entire experimental period.

3.2. Body weight changes

Body weight was recorded at the beginning (day 0), during the experiment (day 5, 10), and post-administration (day 23) to evaluate LC-SPH effects on body weight gain, average weight gain, and feed conversion ratio of each group. The FCR value was calculated as grams of feed consumed per gram of body weight gain. There was no statistical difference in weight gain, Average weight gain, and feed conversion ratio at the dose of 60 and 100 mg/kg body weight. However, LC-SPH significantly decreased ($P < 0.05$) body weight at the dose rate of 20 mg/kg bw 10th-day post-treatment (Table 1).

3.3. Clinical blood parameters

The hematological parameters of chickens with different levels of LC-SPH doses showed in Table 2. There were no statistical differences in observed hematological parameters ($P > 0.05$). Non-significantly decrease observed in WBC at doses 20 mg/kg, 60 mg/kg, and 100 mg/kg body weight.

The effects of different levels of LC-SPH intramuscular injection on the biochemical parameters are shown in Table 3. The calcium value showed a significant decrease ($p < 0.05$) at the dose of 100 mg/kg body weight. However, a slight non-significant decrease in TP and P value were observed in the highest dose group (100 mg/kg bw).

3.4. Gross pathology changes

The necropsy of the different organs, including the heart, liver, lung, and kidneys, revealed no visible macroscopic lesions in the chickens.

3.5. Organ coefficient

The effects of LC-SPH on the organ coefficient are shown in Table 4. The results showed no statistical difference in the heart, liver, spleen, lungs. However, a significant decrease ($p < 0.05$) was observed at the dose of 60 mg/kg in the kidney.

3.6. Histopathology

As shown in Table 4, the dose of 20 mg/kg body weight, the relative weight of heart, liver, spleen, and lungs, was not affected significantly ($P > 0.05$) by LC-SPH. Similarly, LC-SPH caused no histopathological lesions in the heart, muscle, skin, and lungs (Fig. 1).

4. Discussion

Escherichia coli belonging to the family Enterobacteriaceae primarily triggers mammalian intestinal diseases and secondary or local systemic poultry infections, resulting in significant economic losses in the poultry industry [25]. Lincomycin and spectinomycin were evaluated against *Escherichia coli* and *Staphylococcus aureus* in birds, either alone or in various combinations [26]. Clindamycin and spectinomycin combination showed efficacy against *E. coli* and also cause an alteration in biochemical function of kidney, liver, and oxidative stress [27]. The absorption and elimination of intramuscular injection are higher than the oral administration. Abu-Basha et al. [18] stated that spectinomycin has rapid absorption with a maximum concentration of 152.76 lg/mL achieved at 0.25 h and rapidly eliminates. Following intramuscular injection, the present study was conducted related to the safety of the LC-SPH intramuscular injection in poultry.

The metabolism of carbohydrates, proteins, or fats is intimately associated with body performance, body weight, weight gain, feed intake, and feed conversion ratio [28], representing the overall general health status of the animals [29]. 60 mg/kg and 100 mg/kg doses did not cause a significant difference in body weight. Hamdy et al. [26] and Proudfoot et al. [30] found similar results in their studies, suggesting that the combination of lincomycin and spectinomycin does not affect chicken body weight or average weight gain. However, results showed that the 20 mg/kg LC-SPH treated group significantly decreased the bodyweight post-treatment, which may be due to stress during handling of the chickens.

The assessment of hematological parameters can assess the impact of foreign substances on animal blood components, including drugs. It may also describe the roles of chemical compounds related to the blood [31].

Table 1
Effect of LC-SPH (20, 60, and 100 mg/kg body weight) via intramuscular injection for 9 consecutive days on body performance in healthy chickens at 1st and 10th-day post-treatment (ˆX ± SD, n = 10).

Treatment	Dose(x)	15th-day of age			1st-day post treatment			10th-day post treatment		
	Group	BW (g)	AWG (g)	FCR	BW (g)	AWG (g)	FCR	BW (g)	AWG (g)	FCR
LC-SPH	Control	0.50 ± 0.02	0.05 ± 0.01	3.36 ± 0.52	1.12 ± 0.05	0.11 ± 0.02	3.35 ± 0.74	2.04 ± 0.9^{ab}	0.18 ± 0.03	3.23 ± 0.34
	1	0.50 ± 0.02	0.05 ± 0.01	3.30 ± 0.51	1.13 ± 0.07	0.12 ± 0.02	3.33 ± 0.97	1.97 ± 0.12^{b}	0.18 ± 0.03	3.19 ± 0.47
	3	0.49 ± 0.02	0.05 ± 0.01	3.23 ± 0.59	1.11 ± 0.04	0.11 ± 0.02	3.39 ± 1.09	2.05 ± 0.12^{ab}	0.18 ± 0.03	2.81 ± 0.36
	5	0.51 ± 0.02	0.05 ± 0.01	3.22 ± 0.31	1.14 ± 0.07	0.12 ± 0.02	3.50 ± 1.33	2.12 ± 0.12^{a}	0.18 ± 0.04	3.21 ± 0.58
p-value		0.24	0.15	0.79	0.27	0.43	0.96	0.05	0.13	0.12

Significance level (P<0.05), within the column different superscripts are significantly different.
(X) = Times, BW = body weight, AWG = average weight gain, FCR = feed conversion ratio.

Table 2
Effect of LC-SPH (20, 60, and 100 mg/kg body weight) via intramuscular injection for 9 consecutive days on hematological parameters in healthy chickens at 1st-day post-treatment. (ˆX ± SD, n = 10).

Treatment	Dose (x)	WBCs (10^9/L)	RBCs (10^{12}/L)	HGB (g/L)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/L)	PLT (109/L)
LC-SPH	Control	222.0 ± 17.4	2.7 ± 0.5	130.4 ± 21.5	35.6 ± 6.1	133.69 ± 3.6	48.86 ± 2.0	366.00 ± 10.3	35.20 ± 11.2
	1	207.6 ± 9.4	2.4 ± 0.3	115.2 ± 14.8	31.5 ± 4.0	133.52 ± 4.3	48.59 ± 2.0	364.50 ± 5.6	31.60 ± 10.2
	3	216.1 ± 13.1	2.7 ± 0.4	130.0 ± 20.4	35.2 ± 5.4	132.82 ± 3.2	48.86 ± 1.2	368.30 ± 7.3	33.40 ± 9.9
	5	214.7 ± 16.1	2.5 ± 0.5	127.2 ± 26.9	34.3 ± 7.3	133.18 ± 3.2	49.23 ± 1.5	370.20 ± 8.4	28.40 ± 9.5
p-value		0.19	0.36	0.35	0.41	0.95	0.88	0.42	0.50

Significance level (P<0.05), within the column different superscripts are significantly different.

Table 3
Effect of LC-SPH (20, 60, and 100 mg/kg body weight) via intramuscular injection for 9 consecutive days on blood chemical parameters in healthy chickens at 1st-day post-treatment (ˆX ± SD, n = 10).

Treatment	Dose (x)	TP (g/L)	CRE (μ Mol/L)	UREA (mMol/L)	CK (U/L)	Ca (m Mol/L)	P (m Mol/L)	GLO (g/L)	A/G
LC-SPH	Control	31.14 ± 3.72	2.40 ± 0.52	0.29 ± 0.08	4947.10 ± 1205.14	2.58 ± 0.37^{a}	2.09 ± 0.25	17.58 ± 2.85	0.78 ± 0.09
	1	30.07 ± 4.15	1.80 ± 0.63	0.32 ± 0.09	4778.30 ± 1256.15	2.60 ± 0.41^{a}	2.82 ± 1.91	17.11 ± 3.20	0.77 ± 0.09
	3	32.46 ± 5.75	2.60 ± 0.84	0.36 ± 0.15	4129.90 ± 948.41	2.30 ± 0.47^{ab}	2.02 ± 0.30	19.47 ± 4.56	0.75 ± 0.18
	5	29.56 ± 4.40	2.10 ± 0.74	0.28 ± 0.11	4257.50 ± 1165.75	1.96 ± 0.68^{b}	1.85 ± 0.49	16.33 ± 2.73	0.82 ± 0.06
p-value		0.30	0.16	0.67	0.38	0.02	0.18	0.22	0.36

Significance level (P<0.05), within the column different superscripts are significantly different.

Table 4
Effect of LC-SPH (20, 60, and 100 mg/kg body weight) via intramuscular injection for 9 consecutive days on organ coefficient in healthy chickens at 10th-day post-treatment (ˆX ± SD, n = 10).

Treatment	Does (x)	Organ coefficient%				
	Groups	Heart	Liver	Spleen	Lungs	Kidney
LC-SPH	Control	0.52 ± 0.09	1.22 ± 1.27	0.11 ± 0.03	0.50 ± 0.07	0.78 ± 0.06^{a}
	1	0.48 ± 0.05	1.21 ± 1.26	0.21 ± 0.22	0.59 ± 0.08	0.73 ± 0.10^{ab}
	3	0.52 ± 0.07	1.24 ± 1.30	0.13 ± 0.04	0.52 ± 0.13	0.67 ± 0.10^{b}
	5	0.57 ± 0.07	1.21 ± 1.25	0.13 ± 0.03	0.56 ± 0.10	0.74 ± 0.04^{ab}
p-value		0.06	1.00	0.22	0.27	0.05

Significance level (P<0.05), within the column different superscripts are significantly different.

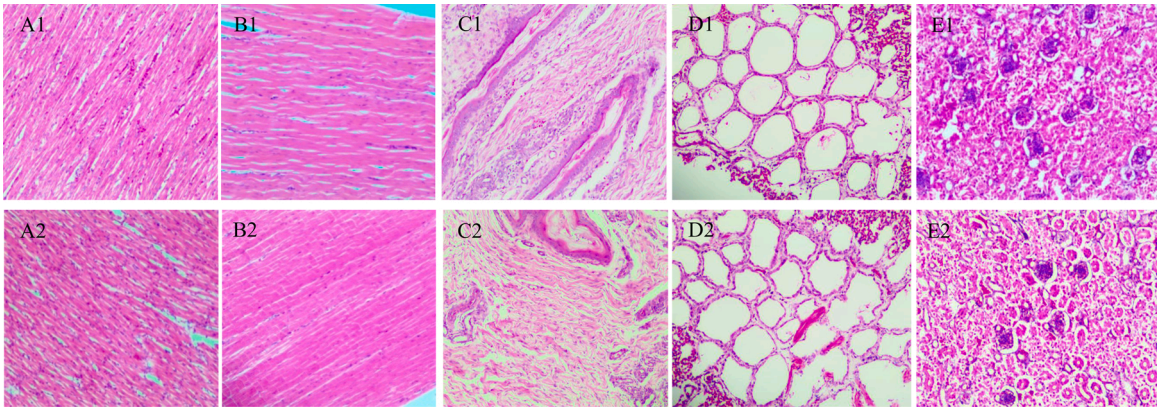

Fig. 1. Photomicrographs of histopathological sections (H & E staining) using 100×. The Control group received no medication (A1, B1, C1, D1, and E1 represent heart, muscle, skin, lung, and kidney, respectively). 100 mg/kg LC-SPH highest dose group (A2, B2, C2, D2, and E2 represent heart, muscle, skin, lung, and kidney, respectively) showed no effects.

WBC, RBCs, HGB, HCT, MCV, MCH, MCHC, and PLT were not significantly affected by LC-SPH at 20 mg/kg, 60 mg/kg, and 100 mg/kg body weight. Yakubu et al. [31] described that increase in WBC implies an increase in immune system activity or might be due to developmental stages variation [32] or an immunological response of the chickens as WBC production is related to phagocytic function [33]. However, the non-significant decrease in WBC may be due to the immune suppression of the chicken by different levels of doses. Selective and localized toxicity indicators of animals, such as HGB, may indicate adverse effects on blood oxygen-carrying capacity, while significant impacts in the HGB value were not observed [34]. The decreased platelet level may harm thrombopoietin [35]. Our results showed a non-significant difference at 20 mg/kg, 60 mg, and 100 mg/kg body weight in platelets level.

Kidney function is important in the toxicity assessment for intramuscular injection because it is essential for the organism's survival [36]. Renal function indices are typically used to determine the normal functions of the different parts of the nephrons [37]. Electrolyte, urea, uric acid, and creatine serum concentrations could provide insight into

the effect of a compound or drug on the kidney's tubular or glomerular portions [23]. Furthermore, the non-effects of 20 mg/kg 60 mg/kg, and 100 mg/kg body weight LC-SPH doses on the renal function indexes may indicate that nephron normal function at the tubular and glomerular level was not affected. Calcium metabolism is similar in avian and mammalian organisms [38]. At 100 mg/kg significant decrease was observed in Ca value. This discrepancy may be due to the interaction of calcium with other minerals reported by Suttle [39], that minerals are well known for interacting with each other in their absorption and metabolism.

Organ indices are relevant parameters in the safety evaluation analysis because they represent the state of organ development in animals [40]. An increase in the organ-body weight ratio indicates inflammation, while a decrease concerns cellular constriction. The non-effect of the high dose group (100 mg/kg) of the LC-SPH on the organ coefficient indicated that the drug did not cause inflammation. It can also justify the non-effect of LC-SPH on the kidney-body weight ratio of the 20 mg/kg body weight group. However, at 60 mg/kg body weight group, the decrease in the kidney parameter observed may be explained by cellular constriction and is considered a minor toxic effect as higher and lower dose groups showed no toxicity [41].

Hence, considering the minor toxicity of 60 mg/kg, our results reveal that intramuscular injection of at least 20 mg/kg body weight has no toxic effects on clinical blood parameters, organ coefficient, growth performance, and histopathological parameters. Thus, a combination of LC-SPH 20 mg/kg body weight i.m injection investigated safe followed daily administration for nine consecutive days in healthy chickens.

5. Conclusion

According to Veterinary international cooperation on harmonization (VICH) guidelines on target animal safety study of veterinary drugs, the combination of LC-SPH injection used at one, three, and five times the recommended dose (20 mg/kg, 60 mg/kg, and 100 mg/kg) body weight to observe the highest level of toxicity in chickens. The results showed that the drug was well tolerated and caused no general, organ, or systemic toxicity post-administration at 20 mg/kg body weight. It is concluded that the experimental results support the safety of 20 mg/kg body weight in combination for the further clinical research study.

Author's contributions

Ejaz Ali Khan: Conceptualization, Writing- Original draft. **Jifei Ma** and **Meng Xiaobin:** Writing- Reviewing. **Yang Jie:** Data collection. **Liang Hong**, **Liu Mengyue** and **Luqman Shah:** Writing and Editing. **Ailing Liu**: Supervision. All the authors have read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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