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Safety Assessment of Ciprofloxacin and Roxithromycin through Haematological and Biochemical Profiling in Poultry

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Abstract

The concurrent therapeutic application of ciprofloxacin and roxithromycin has been suggested as a potential approach for addressing mixed bacterial infections in poultry. The present investigation aimed to determine the hematological and biochemical safety profile of concurrently administered ciprofloxacin and roxithromycin through the oral route in poultry. This study involved a total of sixteen birds, which were divided into two equal but random groups of broiler chickens. In the first group, roxithromycin alone was administered orally for five days. Conversely, the Group II birds received repeated concurrent doses of roxithromycin and ciprofloxacin for consecutive five days adhering to their recommended therapeutic dosages. In both groups, blood samples were collected at various intervals, including before drug administration (0 days), and at 6 hours, 12 hours, 1st day, 2nd day, 3rd day, 4th day, 5th day, 7th day, and 10th day. Following haematological and biochemical analyses, a two-way statistical analysis ($p \leq 0.05$) was conducted utilizing unpaired 't-tests' to compare parameters within the group across various time points and between Groups I and II. The analysis revealed statistically significant differences in haematological parameters such as heterophils, lymphocytes and monocytes, along with one biochemical parameter, specifically aspartate transaminase. Notably, the altered values primarily remained within the normal range and were often attributed to age-related changes, with certain fluctuations being of a temporary nature and reverting to normal levels by the 10th Day. The study findings support the conclusion that the concurrent oral administration of roxithromycin and ciprofloxacin appears to be safe for therapeutic use in poultry.

Keywords: Ciprofloxacin, Haemato-biochemical Evaluation, Poultry, Repeated oral Administration, Roxithromycin

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1. Introduction

Macrolide antibiotics and their semi-synthetic derivatives represent the one of the safest classes of antimicrobial agents extensively employed in the poultry sector[1]. Among these, roxithromycin is a 14-membered ring macrolide semi-synthetic drug synthesized from erythromycin. In roxithromycin's structural refinement, the 9-keto group has been substituted with an etheroxime side chain, a modification designed to enhance its resistance to gastric inactivation. Roxithromycin exhibits bactericidal properties, particularly evident at elevated concentrations[2]. The distinctive kinetic profile of roxithromycin is delineated by its high plasma and intracellular levels, remarkable penetration into bodily tissues and fluids, and an extended half-life conducive to prolonged dosing intervals[3]. Licensed poultry veterinarians have the authorization, according to the United States Food and Drug Administration (US-FDA), to employ macrolides through Extra-Label Drug Use (ELDU), in accordance with the 'Animal Medicinal Drug Use Clarification Act'. Thus, the utilization of roxithromycin in poultry, in an extra-label manner, is permissible[4]. Roxithromycin is extensively utilized in the broiler industry in Asian countries like Thailand where it emerged as one of the most frequently employed macrolide antibiotics and ranked among the top five antibacterial drugs used[5]. Roxithromycin demonstrates notable efficacy against *Mycoplasma* spp., attesting to its substantial usefulness for addressing avian mycoplasmosis[6].

Fluoroquinolones are potent broad-spectrum antimicrobial agents with concentration-dependent bactericidal activity and low MICs against susceptible microorganisms, particularly Gram-negative bacteria. Ciprofloxacin, a second-generation fluoroquinolone, displays favourable oral bioavailability and effective tissue penetration. It is approved for human use and holds promise in veterinary medicine[7,8]. While endorsed for therapeutic poultry use in various Asian and Latin American countries, including Brazil, it lacks approval for food-producing animals in the USA, Europe, Japan, and Australia[9]. In the realm of second-generation fluoroquinolones, ciprofloxacin stands out with its notable susceptibility to Gram-negative bacteria, particularly within the Enterobacteriaceae family. This attribute positions ciprofloxacin as a promising candidate for addressing mixed infections in broiler chickens, especially those encompassing secondary *Escherichia coli* and *Salmonella* species infections[6]. The utility of ciprofloxacin has also been investigated against resistant isolates of *E. coli* recovered from poultry[10]. In field cases of mixed bacterial infections in veterinary medicine where different types of bacteria are present and do not respond to a single treatment, using two antimicrobial drugs together is recommended. The combination of roxithromycin and ciprofloxacin has been advocated for promising treatment for complicated avian mycoplasmosis in poultry, based on the results of the interaction pharmacokinetics of these two antibacterial drugs[6]. The safety of repeated oral doses of ciprofloxacin in poultry has been reported[11], but the safety reports on the use of roxithromycin alone or the co-administration of ciprofloxacin and roxithromycin are lacking in poultry. Thus, the safety implications of administering these two antibacterial agents simultaneously have yet to be documented for this species. Considering this knowledge gap, the present study was undertaken to assess the hematological and biochemical safety of repeated oral doses of roxithromycin alone as well as concurrent use of ciprofloxacin and roxithromycin in poultry.

2. Material and Methods

2.1 Drug and Chemicals

Ciprofloxacin hydrochloride and roxithromycin powders of Indian Pharmacopoeia grade were procured from Shantam Pharmaceuticals Pvt. Ltd., Gandhinagar, Gujarat, as a gift sample. *In vitro* diagnostic kits for biochemical parameters were purchased from Agappe Diagnostic Ltd, Ernakulam, India.

2.2 Poultry

The Institutional Animal Ethics Committee (IAEC) of Veterinary College, Kamdhenu University, Sardarkrushinagar, India, had approved the study protocol. Sixteen male broiler chickens of Cobb strain, aged between 5 to 6 weeks, were employed in the experiment. Before administering the test drugs, the experimental birds were continuously observed during the acclimatization period to ensure the absence of any diseases or abnormalities.

2.3 Experimental Design

Sixteen broiler chickens were randomly divided into two equal groups each having eight birds. Group I birds

were orally administered roxithromycin alone whereas Group II birds were concurrently orally administered ciprofloxacin and roxithromycin for five days.

2.3.1 Drug Administrations

The administration of the drugs involved suspending fine powders of roxithromycin and ciprofloxacin HCl in sterile water as a medium, resulting in a concentration of 20 mg/ml for both drugs. Recommended therapeutic doses based on a pharmacokinetic study[6], *i.e.*, 10 mg/kg body weight once a day for ciprofloxacin and 20 mg/kg body weight twice a day for roxithromycin were administered orally to birds. Roxithromycin was administered twice daily in the morning and evening while ciprofloxacin was administered once daily in the morning using oral gavage.

Table 1. Haematological profiling of Groups I and II birds on various time points (n=8)

Parameters (Unit)	Group	Values of haematological parameters (Mean ± SE)									
		0 h	6 h	12 h	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 10
Hb (g/dl)	I	11.56 ± 0.40	11.40 ± 0.37	11.31 ± 0.38	11.44 ± 0.43	11.49 ± 0.39	11.66 ± 0.35	11.53 ± 0.36	11.83 ± 0.45	11.66 ± 0.35	11.58 ± 0.37
	II	11.65 ± 0.30	11.55 ± 0.42	11.48 ± 0.36	11.73 ± 0.32	11.78 ± 0.39	11.78 ± 0.30	11.91 ± 0.36	11.45 ± 0.28	11.56 ± 0.26	11.76 ± 0.26
PCV (%)	I	35.73 ± 1.30	36.08 ± 1.12	36.33 ± 1.22	36.18 ± 1.45	36.21 ± 1.24	36.74 ± 1.36	36.81 ± 1.25	36.53 ± 1.28	37.16 ± 1.22	36.71 ± 1.16
	II	36.54 ± 0.89	36.63 ± 1.28	36.96 ± 1.40	37.36 ± 1.15	37.45 ± 1.04	37.76 ± 1.02	38.31 ± 1.20	37.59 ± 1.20	37.71 ± 1.01	37.94 ± 0.96
TLC (10^3/ cu mm)	I	23.81 ± 0.60	23.66 ± 0.52	23.86 ± 0.58	24.08 ± 0.51	24.00 ± 0.49	24.61 ± 0.46	24.31 ± 0.48	24.70 ± 0.45	24.79 ± 0.62	25.08 ± 0.60
	II	23.75 ± 0.66	23.71 ± 0.62	24.42 ± 0.59	24.01 ± 0.63	24.35 ± 0.64	24.15 ± 0.68	24.61 ± 0.80	24.72 ± 0.54	25.23 ± 0.59	25.47 ± 0.51
TEC (10^6/ cu mm)	I	2.66 ± 0.08	2.70 ± 0.09	2.56 ± 0.07	2.53 ± 0.07	2.63 ± 0.08	2.71 ± 0.10	2.81 ± 0.10	2.72 ± 0.08	2.84 ± 0.08	2.91 ± 0.08
	II	2.53 ± 0.07	2.56 ± 0.11	2.59 ± 0.09	2.63 ± 0.08	2.59 ± 0.08	2.66 ± 0.09	2.67 ± 0.09	2.77 ± 0.10	2.84 ± 0.11	2.83 ± 0.10
Heterophils (%)	I	26.50 ± 1.20	26.38 ± 1.31	24.63 ± 0.42#	24.38 ± 0.71	25.50 ± 0.82	24.88 ± 0.74	25.25 ± 0.80	25.00 ± 0.65	26.13 ± 0.90	26.88 ± 0.85
	II	23.50 ± 0.68	25.00 ± 0.93	26.50 ± 0.80#	26.88 ± 0.95	25.38 ± 1.05	25.13 ± 0.69	25.13 ± 1.22	24.25 ± 0.73	25.38 ± 0.60	25.25 ± 1.01
Lymphocytes (%)	I	65.25 ± 1.92	66.38 ± 1.61	68.38 ± 1.15	67.50 ± 1.28	66.50 ± 1.21	67.00 ± 0.98	66.75 ± 1.22	66.38 ± 1.43	65.63 ± 1.40	65.75 ± 1.03
	II	69.75 ± 1.10	67.63 ± 1.08	64.75 ± 0.94	64.63 ± 1.84*	67.38 ± 1.59*	67.75 ± 1.41	67.88 ± 1.79	69.00 ± 1.04	65.50 ± 0.82	67.00 ± 1.45
Monocytes (%)	I	5.38 ± 0.68	4.00 ± 0.33*	4.25 ± 0.73	5.13 ± 0.61	4.88 ± 0.85	5.38 ± 0.71	4.88 ± 0.64	5.50 ± 0.85	5.63 ± 1.07#	4.50 ± 0.71
	II	4.13 ± 0.48	4.25 ± 0.53	5.38 ± 0.63	5.88 ± 0.85*	4.63 ± 0.78	4.25 ± 0.75	4.25 ± 0.73	4.38 ± 0.65	5.38 ± 0.53#	4.63 ± 0.86
Eosinophils (%)	I	2.88 ± 0.55	3.25 ± 0.25	2.75 ± 0.59	3.00 ± 0.42	3.13 ± 0.40	2.75 ± 0.25	3.13 ± 0.35	3.13 ± 0.55	2.63 ± 0.56	2.88 ± 0.30
	II	2.63 ± 0.32	3.13 ± 0.58	3.38 ± 0.53	2.63 ± 0.46	2.63 ± 0.26	2.88 ± 0.40	2.75 ± 0.37	2.38 ± 0.32	3.75 ± 0.49	3.13 ± 0.40

Hb: Haemoglobin; PCV: Packed Cell Volume; TLC: Total Leucocyte Count; TEC: Total Erythrocyte Count.

*Values having *superscript in the same row indicate a significant difference at $p \leq 0.05$ when compared with the control (0 h) values.*

Values having #superscript indicate a significant difference at $p \leq 0.05$ at the same time point between the two groups for specified parameters.

Table 2. Biochemical profiling of Groups I and II birds on various time points (n=8)

Parameters (Unit)	Group	Values of biochemical parameters (Mean ± SE)									
		0 h	6 h	12 h	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 10
ALT (U/L)	I	7.31 ± 0.42	7.11 ± 0.33	6.96 ± 0.25	7.69 ± 0.54	7.71 ± 0.55	7.54 ± 0.45	7.68 ± 0.54	7.38 ± 0.49	6.76 ± 0.38	7.08 ± 0.44
	II	7.44 ± 0.38	7.28 ± 0.51	7.28 ± 0.47	7.75 ± 0.44	7.54 ± 0.60	8.40 ± 0.76	8.21 ± 0.63	7.79 ± 0.51	7.76 ± 0.36	7.71 ± 0.35
AST (U/L)	I	193.19 ± 5.74	198.23 ± 5.17	190.45 ± 3.05	197.44 ± 4.81	192.76 ± 3.35$^{\#}$	188.14 ± 2.80$^{*\#}$	191.49 ± 3.99	194.35 ± 2.88*	185.21 ± 2.61*	189.96 ± 3.50
	II	189.71 ± 3.60	194.31 ± 4.41	195.36 ± 4.17	190.14 ± 3.71	199.99 ± 5.42$^{*\#}$	203.23 ± 7.35$^{*\#}$	197.78 ± 6.67	190.91 ± 4.10	190.84 ± 2.64	190.98 ± 3.82
ALP (U/L)	I	711.25 ± 0.40	706.39 ± 0.37	708.61 ± 0.38	702.56 ± 0.43	701.14 ± 0.39	704.15 ± 0.35	705.41 ± 0.36	695.24 ± 0.45	702.21 ± 0.35	711.19 ± 0.37
	II	701.71 ± 32.35	705.04 ± 31.68	708.58 ± 32.04	689.70 ± 34.69	693.86 ± 29.93	693.81 ± 36.50	692.34 ± 31.31	687.59 ± 30.77	695.93 ± 31.50	692.19 ± 32.99
ACP (U/L)	I	3.69 ± 0.10	3.66 ± 0.10	3.68 ± 0.09	3.69 ± 0.11	3.69 ± 0.12	3.63 ± 0.11	3.68 ± 0.10	3.70 ± 0.12	3.70 ± 0.11	3.74 ± 0.12
	II	3.76 ± 0.14	3.70 ± 0.14	3.73 ± 0.14	3.69 ± 0.12	3.73 ± 0.11	3.69 ± 0.14	3.76 ± 0.09	3.74 ± 0.13	3.71 ± 0.13	3.73 ± 0.10
Blood glucose (mg/dl)	I	255.88 ± 3.11	253.25 ± 4.46	250.13 ± 3.35	253.38 ± 4.07	245.63 ± 5.71	247.88 ± 4.57	252.13 ± 4.04	248.88 ± 3.49	252.00 ± 4.17	257.88 ± 3.47
	II	260.75 ± 5.30	264.25 ± 8.22	251.88 ± 6.15	260.25 ± 7.27	250.88 ± 4.70	250.75 ± 5.69	260.88 ± 7.43	251.38 ± 4.04	259.38 ± 5.77	259.75 ± 5.99
TBIL (mg/dl)	I	0.13 ± 0.00	0.13 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
	II	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.00
Creatinine (mg/dl)	I	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
	II	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
BUN (mg/dl)	I	1.44 ± 0.13	1.43 ± 0.13	1.40 ± 0.14	1.38 ± 0.15	1.40 ± 0.14	1.38 ± 0.15	1.38 ± 0.15	1.36 ± 0.13	1.44 ± 0.16	1.43 ± 0.17
	II	1.73 ± 0.17	1.69 ± 0.17	1.76 ± 0.15	1.74 ± 0.20	1.60 ± 0.15	1.60 ± 0.18	1.70 ± 0.17	1.64 ± 0.18	1.68 ± 0.14	1.69 ± 0.16
Total Protein (g/dl)	I	3.80 ± 0.07	3.83 ± 0.05	3.80 ± 0.08	3.91 ± 0.08	3.99 ± 0.08	3.95 ± 0.09	3.96 ± 0.07	3.90 ± 0.06	3.93 ± 0.04	3.98 ± 0.06
	II	3.75 ± 0.06	3.75 ± 0.07	3.76 ± 0.08	3.81 ± 0.05	3.83 ± 0.05	3.80 ± 0.05	3.79 ± 0.08	3.85 ± 0.07	3.85 ± 0.07	3.86 ± 0.07
Albumin (g/dl)	I	1.41 ± 0.04	1.36 ± 0.03	1.38 ± 0.04	1.38 ± 0.02	1.36 ± 0.04	1.41 ± 0.04	1.36 ± 0.03	1.34 ± 0.03	1.38 ± 0.04	1.41 ± 0.04
	II	1.39 ± 0.03	1.38 ± 0.02	1.35 ± 0.03	1.39 ± 0.03	1.39 ± 0.02	1.39 ± 0.03	1.41 ± 0.04	1.36 ± 0.03	1.35 ± 0.02	1.40 ± 0.02

ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase; TBIL: Total Bilirubin; BUN: Blood Urea Nitrogen.

*Values having *superscript in the same row indicate a significant difference at $p \leq 0.05$ when compared with the control (0 h) values.*

Values having $^{\#}$superscript indicate a significant difference at $p \leq 0.05$ at the same time point between the two groups for specified parameters.

2.3.2 Sample Collection

Blood samples were obtained from the treated birds at specified time intervals by drawing blood from wing veins. For hematological analyses, the blood was collected into K_3EDTA tubes, and for plasma biochemical analysis, it was collected into clean and dried heparinized tubes. Blood was collected from wing veins at various time points: initially at 0 hours (considered as the control sample before drug administration), followed by 6 and 12 hours, and subsequently once a day on days 1, 2, 3, 4, 5, 7, and 10. For assessing hematological parameters, 0.5 to 0.8 ml of blood was collected, while 1.0 to 1.2 ml of blood was drawn to harvest the plasma. The plasma samples intended for biochemical analysis were transferred to cryovials and temporarily stored at -20°C until analysis.

2.4 Haemato-Biochemical Analysis

For the assessment of hematological parameters such as Hemoglobin (Hb), Packed Cell Volume (PCV), Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC), and Differential Leukocyte Count (DLC) in poultry blood, standard protocols[12,13] were followed, and the analyses were conducted manually. Plasma samples were used to measure biochemical parameters using the RX Monaco, an automated clinical chemistry analyzer (Randox Laboratories Ltd., United Kingdom). Acid phosphatase levels were assessed using a semi-automatic biochemistry analyzer (RX-50V, Microlab, India) with a commercially available kit from Crest Biosystems, Goa, India.

2.5 Clinical Observations

The treated birds were monitored at intervals: every four hours on the first day, three times a day for the following four days, and twice a day for the subsequent five days. This was done to detect any clinical signs, physical abnormalities, or allergic reactions if they occurred. Additionally, their behaviour, nervous alertness, and gait were noted during the observation.

2.6 Statistical Analysis

Safety data obtained from haematological and biochemical analyses were statistically assessed within the group, examining parameters at multiple time points ($p \leq 0.05$) and a comparison was also made between Groups I and II using the 'unpaired (independent) t-test,' conducted using SPSS ver. 19 software.

3. Results

3.1 Haematological Alterations

Significant statistical variances were identified in the mean hematological parameters, specifically the monocyte count at 6 hours in Group I birds and on the 1st day in Group II birds, when compared to their respective control mean values (Table 1). Statistical differences were also noted in the mean lymphocyte count of Group II birds on the 1st and 2nd days, relative to the pre-dosing (control) values (Table 1). Heterophil count at 12 hours and monocyte count on the 7th day exhibited significant variations between Groups I and II.

3.2 Biochemical Alterations

Among the analyzed parameters, a statistically significant alteration was detected only in the Aspartate Aminotransferase (AST) levels. The mean AST values showed significant disparities on the 3rd, 5th, and 7th days for Group I birds, and on the 2nd and 3rd days in Group II birds when compared to their respective control mean values (Table 2). A statistical difference in AST values on the 2nd and 3rd day was found upon comparing the mean values of Groups I and II birds.

3.3 Clinical Examinations

Routine monitoring at scheduled intervals revealed an absence of drug-induced adverse reactions in any of the treated birds. Fecal excreta consistency remained normal throughout the experimental period. No clinical signs or physical abnormalities were observed, and the gait of the birds remained normal. Birds exhibited normal behaviour and alertness, demonstrating an overall unremarkable clinical profile during the experimental duration.

4. Discussion

The therapeutic use of two antibacterial drugs to combat challenging mixed infections or achieve additional therapeutic effects is a common practice in poultry farming. Such a suggested combination consists of ciprofloxacin, a fluoroquinolone, and roxithromycin, a macrolide compound. This combination is suggested to treat Complex Chronic Respiratory Disease (CCRD) in broiler chickens, which is caused by mixed infections

of *Mycoplasma gallisepticum* and *Escherichia coli*[6]. Roxithromycin has been reported to exhibit effectiveness in high doses against *Mycoplasma gallisepticum* and is could be employed in an extra-label manner in poultry. Considering the rising challenge of resistance seen in established anti-mycoplasma drugs like tylosin, there is potential for repurposing roxithromycin to target mycoplasma infections in the poultry. Safety data concerning the utilization of roxithromycin in elevated therapeutic doses, either as a standalone treatment or in combination with other antibacterial drugs, remains absent in poultry or any other species. Consequently, the findings of this study offer valuable insights into the hematological, biochemical, and clinical safety profiles associated with roxithromycin, as well as the co-administration of ciprofloxacin and roxithromycin in poultry. In the present study, drug dosing was done for five days, aligning with the typical therapeutic dosage regimen for broiler chickens. However, blood samples were collected over a span of 10 days. Thus, the samples were collected up to the fifth day after the administration of the last oral dose to the birds, to assess whether there was a reversal of haemato-biochemical alterations after discontinuation of dosing.

The normal values of haematological parameters *viz*., haemoglobin (10.2-15.1 g/dl), RBC count (2.5 -3.9 x 10^{12}/L), WBC count (1.9-9.5 x 10^{6}/L), and PCV (30-49 %) in adult domestic fowl (*Gallus domesticus*) has been reported[13]. However, these biological values are highly variable and differ from breed to breed and strain to strain, as well as depend upon multiple factors like age, sex, physiological and nutritional status[14]. The biochemical parameters in indigenous chickens and broiler chickens in various regions of the world also differ[15,16]. Haematological parameters have definite patterns of change as the advancing age in birds[13]. Similarly, the values of biochemical parameters including total protein, glucose, creatinine, and albumin in broilers, are also known to be affected by age[17]. Therefore, pre-dose values of parameters were taken as control as well as a reference for interpreting the safety data and correlated with the clinical evidence of health monitoring.

Following dosing of roxithromycin alone at high therapeutic doses (Group I birds), no statistically significant difference was observed in the values of any haematological parameters studied except monocyte count at 6 hours only and not at later time points (Table 1). There was a non-significant increase in values of PCV and TLC, which too was found to be within the normal range. In addition, the maximum individual value of PCV was 43.2%, which is lower than the benchmark of dehydration status (PCV values > 56 %) in poultry. In birds, TLC shows an increasing trend from 1 week to 7 weeks of age[13]. The increase in TLC values in the present study seems to be simply age-associated as there is a 7-10-day difference between pre-dose and the 7th and 10th-day samples.

Statistically significant differences were observed in only one biochemical parameter namely AST at 3rd, 5th and 7th day in Group I and at 2nd and 3rd day in Group II birds. However, their values returned to normal by the 10th day (Table 2). Similar to our findings, the repeated oral administration of the related macrolide drug clarithromycin at a dosage of 7.5 mg/kg body weight, daily for 5 consecutive days resulted in significant increases in AST levels in Ross broiler chickens[18]. Aspartate Transaminase (AST), alternatively referred to as Glutamic-Oxaloacetic Transaminase (sGOT), serves as a prevalent biochemical indicator for assessing the degree of liver and skeletal muscle damage as well as inflammation in both animals and avian species. There was a non-significant increase in total protein levels which is likely attributed to the protein-rich feed commonly provided to broiler chickens as dietary components that can influence the blood profile of healthy poultry birds[19]. In contrast, tilmicosin, a macrolide antibiotic, is reported to induce significant reductions in total protein and albumin levels in broiler chickens; however, these alterations were transient and subsequently normalized[20]. In comparison to other antimicrobial agents, macrolides are regarded as relatively safe, with adverse effects typically confined to gastrointestinal disturbances, such as diarrhea. However, no such clinical signs were observed in the present study.

Upon the co-administration of ciprofloxacin and roxithromycin (Group II), significant differences were observed in the mean values of hematological parameters such as monocyte and lymphocyte counts (Table 1) and a biochemical parameter i.e. AST (Table 2). A short-term change in the lymphocyte count was observed at intermediate time points of 1st and 2nd day. Like the Group I birds, the mean values of parameters like PCV, TLC, and TEC also demonstrated a general non-significant increasing trend in Group II birds. This trend seems to be associated with the factor of age. Biochemical parameters such as AST displayed statistically higher

values at intermediate time points, which hold no clinical-pathological significance and returned near to mean control value on the 10th day, suggesting a transient nature. Comparable patterns of total protein elevation were noted in both Group I and Group II birds, seemingly associated with dietary factors. The milder hypoglycemia observed in the co-treated Group II birds could potentially be attributed to ciprofloxacin. Ciprofloxacin is known to cause transient drug-induced hypoglycemia in broiler chickens[11]. There were no scientific reports available regarding the safety of combining macrolides and fluoroquinolones in animals or birds, thus precluding a comparison of these results.

Statistical comparison between the Groups I and II birds revealed significant differences only for heterophil count on 12 hours, monocyte count on day 7 and AST values on 2nd and 3rd day only. However, values of these parameters at said time points were within the normal range and returned near to the control values on the 10th day, after discontinuation of the dosing on the 5th day. Regarding clinical observations, the results of our study indicated that the broiler birds evaluated for safety, both in Groups I and II, did not display any physical abnormalities, gait issues, or behavioural problems during the entire experiment. The health conditions of the treated birds were assessed as satisfactory, considering their regular behaviour, growth rate, and the absence of noticeable clinical signs or symptoms.

5. Conclusion

In conclusion, the changes observed in certain hematological and biochemical parameters during the safety assessment did not exceed the normal ranges of those parameters. Most of the alterations in parameters were nonsignificant and largely attributed to the birds' natural ageing process and reversible upon discontinuation of dosing. Based on the findings from hematological, biochemical, and clinical evaluations in this study, it is inferred that using ciprofloxacin and roxithromycin concurrently at oral doses of 10 and 20 mg/kg body weight, respectively, over five consecutive days in poultry is likely to be clinically safe.

6. Acknowledgement

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