Journal of Current Veterinary Research

ISSN: 2636-4026

Journal home page: http://www.jcvr.journals.ekb.eg

Pharmacology

Potential Effect of Cefotaxime on Kinetic of Apramycin in *E. coli* Infected Broiler Chickens

Hanem F. El-Gendy, Eman Elhusseiny, Saber El Hanbally*,

Department of Pharmacology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

**Corresponding author: Saber.ahmed@vet.usc.edu.eg* *Received: 1/12/2024 Accepted: 27/1/2025*

ABSTRACT

The effect of cefotaxime on kinetics of apramycin in *E. coli* infected broilers. The concentration of apramycin in serum was measured with (HPLC) high performance liquid chromatography. Apramycin's pharmacokinetics was described via a two-compartment open model. Serum concentration and kinetic of apramycin after 10 mg/kg b.wt. single IV injection and IV injection of 10 mg/kg b.wt. of apramycin concurrent with IV injection of 10 mg/kg b.wt. of cefotaxime with a single dose, showed that apramycin was detected in serum till 24 hrs with mean rate 0.200± 0.015 µg/ml and till 12 hrs 0.040± 0.009 µg/ml of infected chickens, with ($t_{0.5\alpha}$) of 0.18 ± 0.01 h and 0.14± 0.01h, ($t_{0.5\beta}$) of 4.27± 0.29 h and 1.46± 0.07h, (CL_{tot}) was 0.10± 0.01L/kg/h and 0.67± 0.04L/kg/h, {V_{dss}} was 1.11± 0.05L/kg and 1.08± 0.02L/kg in infected chickens respectively. Serum concentration and kinetic of single oral intake of 25 mg/kg b.wt. of apramycin and a single oral intake of 25 mg/kg b.wt. of apramycin concurrent with single IM injection of 10 mg/kg b.wt. of cefotaxime, indicated that apramycin was detected till 8 hrs with mean rate 0.633±0.021 µg/ml and 0.550±0.016 µg/ml in serum of infected chickens, respectively, with C_{max} 3.273± 0.10 µg/ml and 2.277 ± 0.025 µg/ml at T_{max} (2.29± 0.06 h and 2.456± 0.005 h, ($t_{0.5\alpha}$) was 0.81± 0.14h and 1.421± 0.009 h, {$t_{0.5\ (\beta)}$} was 1.65± 0.11h and1.414± 0.009 h, AUC 15.25± 0.63 µg/h/ml and 12.661± 0.200 µg/h/ml, bioavailability% was 12.04±0.4%, 33.31±0.32% in infected chickens respectively.

Keywords: Apramycin, Cefotaxime, kinetic, Bioavailability, Broilers.

1. INTRODUCTION

Aminoglycosides are powerful bactericidal antibiotics that suppress protein production by binding with great sympathy to the A-site of the 16s ribosomal RNA of the 30s ribosome (Kotra et al., 2000). They are active against a diversity of Gram+ve and Gram-ve pathogens. Aminocyclitol is found as a product of aminoglycoside antibiotics, commonly known as pseudo sugars or pseudo saccharides. Aminocyclitols are chemically composed of a carbon ring with an amine functional group.

Apramycin is a bactericidal aminocyclitol antibiotic. Since the early 1980s, it has been utilized in veterinary medicine, and was accepted in 1999 in China (Zhang *et al*., 2009). It is produced from a certain bacteria (*Streptomyces tenebrarius*). It is fundamentally related to aminoglycoside group of antibiotic but differs from the parent compound consisting of a core aminocyclitol moiety and different kinds of sugars. It is defined by a 4-amino-4-deoxy-D-glucose moiety, a glycosidic linkage 1-1', and an octadiose (Walton *et al*., 1978; Tatsuta *et al*., 1984). It is used to delicacy intestinal and systemic infections. It is in effect against a variation of gram-ve bacteria, comprising (*E. coli, Proteus, Salmonella, Pseudomonas, Klebsiella, Pasteurella, Bordetella bronchiseptica, and Treponema hyodysenteriae*) also, it is effective against *Mycoplasma spp.* and *Staphylococcus spp*. It reacted against *Salmonella spp*. and *E. coli* cultures *in vitro* that exhibit resistance to dihydrostreptomycin and neomycin, and is not well absorbed by animals' digestive systems. Apramycin is utilized to treat *E. coli* septicemia in poultry, colibacillosis and salmonellosis in calves, colibacillosis in lambs, bacterial enteritis in pigs, and. It was also given to rabbits (Elbadawy and Aboubakr, 2017). It suppresses protein production by attaching to the ribosomal 30s subunits irreversibly. According to current theories, apramycin inhibits peptide chain elongation through binding to the 30s subunit of ribosome 16 S rRNA A-decoding site, causing the combination of non-cognate amino acids via encouraged miscoding action (Riedel *et al*., 2019).

Cefotaxime was the first third-generation, semisynthetic, cephalosporin to be widely available on the markets, and administered intramuscularly or intravenously (Pacifici & Marchini, 2017). Spectrum of activity of cefotaxime is broad that has a strong resistance to the -lactamase enzyme's effect (Xu *et al*., 2020). *In vitro*, cefotaxime has an excellent bactericidal wide spectrum of action against Gram-ve aerobic, anaerobic, and Gram+ve bacteria. It is just by way of active as benzyl penicillin against *Streptococcus pneumoniae* and pyogenes, it is also quite effective against penicillin-resistant and drug-resistant strains of *Streptococcus pneumoniae* and weak activity against *enterococci*, particularly *Streptococcus faecalis*. (Varghese, *et al*., 2022). Until now, no information about the pharmacokinetic profile between apramycin and cefotaxime in broiler chickens is recorded. The available literatures are on the pharmacokinetic of apramycin and kanamycin (Lashev *et al*., 1992), kinetic of apramycin and sulphaclorpyridazine Co administered with avoparcin and flavophospholipol to chickens (Lashev *et al*., 1998), pharmacokinetics gentamicin alone and combination with paracetamol in buffalo calves (Baxla *et al*., 2010), relation bioavailability & Pharmacokinetics of an oral apramycin-amoxicillin mixture in pigs (Dai *et al*., 2017), Influence of dose escalation and method of administration on tissue residues profile and withdrawal time of gentamicin and apramycin in broiler chickens (Hesham *et al*., 2019).

The goal after the current inquiry was to evaluate bioavailability and the pharmacokinetic of apramycin alone and concurrent administration with cefotaxime in infected broiler chickens.

2. MATERIAL AND METHODS

2.1. ***Antibiotics***

Apramycin: it was gained from WAKI Pharma for pharmaceutical industries under trade name (Apracure ®). It is intended for oral use. Each 100 grams includes 86.5 grams of apramycin sulfate, which is equivalent to 59.5 grams of apramycin base.

Cefotaxime: it was obtained from EVA Pharma Egypt, under trade name (Cefotaxime ®), it is a powder for solution for IV or IM injection .Vial contain cefotaxime sodium 2.097g equivalent to cefotaxime 2g. The powder was dissolved in distilled water immediately before injection.

2.2. Birds

Sixteen experimentally *E.coli* infected broiler chickens of 4 weeks of age, weighing from 1500-1800 g. the birds infected with *E.coli* O 78. *Escherichia coli* O78 was obtained from serology unit in Animal Health Research Institute. Infection occur by injection of 0.5 ml of requisite concentration (10^7micro organism /ml) subcutaneous in the neck region of birds .Two days post infection, appearance signs of depression, off food, bloody diarrhea and difficult breathing with severe PM 1eslons (air saculitis, pericarditis and per hepatitis) and high mortality rate (30 % nearly 3 chickens) (El. Sayed *et al*., 2018). The experimental procedures complied with the Guidelines for Animal Experimentation and received approval from the Ethical Committee at the Faculty of Veterinary Medicine, University of Sadat City, Egypt. Management of the animals was directed in agreement with the recommendations and rules set forth by Animal Care House (approval No, VUSC-026-1-24).

2.3. Experimental design

Group I: it comprised 8 infected chickens, which administered IV single dose (10 mg/kg b.wt.) apramycin in wing vein and then after 15 days the unchanged birds were given oral a single dosage of 25 mg/kg b.wt. apramycin.

Group II: it included 8 infected chickens, which administered a single IV dosage of 10 mg/kg b.wt. apramycin in the wing vein at right side and a single IV of 10 mg/kg b.wt. cefotaxime in the wing vein at left side. These birds were kept for 15 days following IV injection to guarantee the complete apramycin elimination and cefotaxime from their systems. Subsequently, they received oral single dose of 25 mg/kg b.wt. of apramycin, in addition to a 10 mg/kg b.wt. IM dose of cefotaxime to evaluate the bioavailability of apramycin.

2.4. Samples

Each bird's wing vein was used to draw 1 ml of blood at 5, 15, and 30 minutes as well as 1, 2, 4, 6, 8, 12, and 24 hrs after antibiotic was managed. After permitting blood samples to clot at room temperature, the clear sera were obtained by centrifuging the samples for 15 mins at 3000 r.p.m. Prior to HPLC analysis, the resultant sera were kept at -20° C in sterile plastic eppendorf tubes.

2.5. Analytical approaches.

Apramycin levels in the blood were measured using high-performance liquid chromatography. Plasma samples of 0.5 mL were mixed with 1.0 mL of 10% trichloroacetic acid solution containing 0.04 mM Na2EDTA. After vortexing for two minutes, the samples were centrifuged for ten minutes at 4°C and 9500 ×g. The clear supernatant was transferred to a 5 mL centrifuge tube, and the extraction process was repeated

to collect the supernatant for the purification and enrichment procedure (Dai *et al*., 2017). The sample was prepared for analysis as follows: First, a methanol and water solution was used to condition the Oasis MCX 30 mg, 1 cc cartridge. Then, the supernatant was collected, and the cartridge was washed with water and a 0.5% ammoniated methanol solution. The cartridge was dried, and the analytes were eluted with a 5% ammoniated methanol solution. The elute was evaporated under nitrogen at 50°C, and a borate buffer solution (0.012 M, pH 9.0) was added and vortexed for 1 minute. Next, a FMOC-Cl (2.0 mM in acetonitrile) solution was added and vortexed for 30 seconds, and the mixture was left in the dark at room temperature for 20 minutes. Glycine (0.1 M) was then added to stop the reaction. The final mixture was centrifuged, and 20 µL of the supernatant was transferred to an auto-sampler vial for HPLC analysis (Dai *et al*., 2017). High-performance liquid chromatography (HPLC) was performed using an Agilent 1200 system with a reversed-phase column (C18, 4.6 x 250 mm, 5 µm, Agilent, USA). An Agilent fluorescence detector was used for detection, with an excitation wavelength of 260 nm and an emission wavelength of 315 nm. The mobile phase for separation was a mixture of acetonitrile and water (77:23, v/v), with a flow rate of 1.0 mL/min, and the column oven temperature was maintained at 30 ± 5°C.

Calibration charts for apramycin were created using a series of blank plasma samples with eight different concentrations (0.05, 0.1, 0.25, 0.5, 1, 5, 10, 25, and 50 µg/ml), resulting in a correlation coefficient (r2) of 0.998. **Figure (1)**.

The boundary of determination for apramycin was 0.015 µgmL and the edge of quantification was 0.05 µgmL for the serum. The RSD% of intra-day for APR was 5.34 and the rate for inter-day was 5.66. Whereas the recovery rate % range from (88% -110.9%).

2.6. Pharmacokinetic analysis

The software program Phoenix Win Non-lin 2.1 (Pharsight analysis by the USA) was used to conduct a two-compartmental pharmacokinetic analysis of the data. Software tools were used to estimate pharmacokinetic variables and fit data from each bird independently. The distribution, absorption, and elimination half-lives ($t_{0.5\alpha}$, $t_{0.5a}b$, $t_{0.5\beta}$, $t_{0.5el}$), extrapolated zero-time intercepts (A and B), first order absorption and elimination rate constants (K_{ab} and K_{el}), distribution and elimination phase hybrid rate constants (α and β), and transfer rate constants (K_{12} and K_{21}). We calculated the maximum serum concentration (C_{max}), time to reach (T_{max}), mean time of residence (MRT), and AUC 0-∞. The steady-state distribution volume (V_{dss}), central compartment (Snedecor, 1969).

3. RESULTS

Following apramycin IV single injection in infected birds

Table 1 displayed antibiotic average sera concentrations over various time periods. After IV injection, the serum concentration-time curve of apramycin revealed that the medication followed a two-compartment open model, as shown in **Figure 2**. **Table 2** displayed the apramycin's computed pharmacokinetic parameters.

With a half-life of distribution ($t_{0.5\alpha}$) of 0.18 ± 0.01 h and half-life of elimination (t0.5β) of 4.27± 0.29 h, the drug was

distributed in the birds at a rate of 3.93± 0.29 h^{-1}. The CL_{tot}, or total body clearance, was 0.10±0.01 L/kg/h. The apparent volume of distribution (V_{ss}) in the current study was 1.11 ±0.0501 L/kg, while the steady-state volume of distribution (V_{dss}) was 0.423 ± 0.008 L/kg. The transfer of apramycin from the central to peripheral compartment occurred more quickly (K_{12} = 2.22± 0.16 h^{-1}) than the transmission from the peripheral to the central compartment (K_{21} = 1.40± 0.16 h^{-1}).

After oral intake of 25 mg/kg b.wt of apramycin in infected chickens

Table 1 displayed the drug's mean serum concentrations over various time periods. **Table 2** displayed the apramycin's computed pharmacokinetic parameters. Two hours after oral administration, the average top sera level (3.235 ±0.078 µg/ml) was reached. After taking apramycin 25 mg/kg.b.wt. orally once, the drug's serum concentrations extreme were reached at a time of maximum concentration T_{max} (2.29± 0.06 h) of administration and C_{max} (3.273± 0.10 µg/ml). The obtained results showed that apramycin was poorly absorbed after oral administration, with an absorption half-life {$t_{0.5\ (ab)}$} of 0.81± 0.14 h and an apparent first order absorption rate constant {K_{ab}} of 0.99± 0.16 h^{-1}. The elimination half-life {$t_{0.5\ (\beta)}$} was 1.65± 0.11 and the rate {K_{el}} of apramycin was 0.43± 0.03 h^{-1}.

The calculated bioavailability (F%) of apramycin in infected birds after oral intake of 25 mg/kg b.wt of apramycin was 12.04±0.4**%.**

After single IV injection of apramycin concurrent with IV injection of cefotaxime in infected broiler chickens

Six infected broiler chickens received IV injections of cefotaxime (10 mg/kg b.wt.) and apramycin (10 mg/kg b.wt.). **Table 3** displayed the average drug serum concentrations over various time periods. After intravenous injection, the serum concentration-time curve of apramycin revealed that the medication followed a two-compartment open model, as shown in **Figure 3**. **Table 4** contains the apramycin's computed pharmacokinetic parameters. The half-life of elimination {$t_{0.5\ (\beta)}$} was 1.65 ± 0.11 and the rate {K_{el}} of apramycin was 0.43 ± 0.03 h^{-1}. The drug was distributed {α} equal to 5.08 ± 0.27h^{-1} in the body with a distribution half-life ($t_{0.5\alpha}$) of 0.14 ± 0.01h, The elimination half-life ($t_{0.5\beta}$) of 1.46 ± 0.07 hours, The total body clearance (CL_{tot}) was 0.67 ± 0.04 L/kg/h. Apramycin was transferred from central to peripheral compartment at a faster rate (K12 = 2.42 ± 0.15 h^{-1}) than its path from peripheral compartment to central compartment (K21 =1.55 ± 0.09h^{-1}). In the current study the apparent volume of distribution was 0.421 ± 0.007 where volume of distribution at steady – state {V_{dss}} was 1.08± 0.02 L/kg, this value.

Following oral administration of apramycin concurrent with IM injection of cefotaxime in infected broiler chickens.

After 15 days the same infected chicken which injected intravenously of 10 mg/kg b.wt. of apramycin and IV injection 10 mg/kg b.wt. of cefotaxime, taken 25 mg/kg b.wt. of apramycin and IM injection 10 mg/kg b.wt. of cefotaxime.

The mean serum concentrations of the drug at different time intervals were shown in **Table 3 & Figure 3**. The calculated pharmacokinetic parameters of apramycin were recorded in **Table 4.**

The average highest serum level (2.297 ± 0.014 µg/ml) attained 2 hours post oral handling. Following a single oral administration of apramycin 25 mg/kg.b.wt. and IM injection 10 mg/kg b.wt. of cefotaxime in infected group, the drug got its maximum serum concentrations C_{max} (2.277 ± 0.025 µg/ml) at maximum time concentration T_{max} (2.456± 0.005 hours) of administration. The obtained results revealed that apramycin was poorly absorbed afterward its oral intake with an apparent first order absorption rate constant $\{K_{ab}\}$ of 0.488 ± 0.003$h^{-1,}$ while half-life of absorption $\{t_{0.5\ (ab)}\}$ was 1.421± 0.009 h.

Apramycin was rate of elimination $\{K_{el}\}$ equal to 0.490 ± 0.003 h^{-1} and the elimination half-life $\{t_{0.5\ (\beta)}\}$ was 1.414± 0.009 h. The calculated AUC was found to be 12.661± 0.200 µg/h/ml.

The calculated bioavailability (F%) of apramycin in infected broiler chickens after oral administration of 25 mg/kg b.wt of apramycin and IM injection 10 mg/kg b.wt of cefotaxime was 33.31±0.32%.

Figure 4 & 5 illustrated the effect of cefotaxime on apramycin serum concentrations in infected broilers after (IV apramycin, IV apramycin with IV cefotaxime) and (oral apramycin, oral apramycin with IM cefotaxime), respectively.

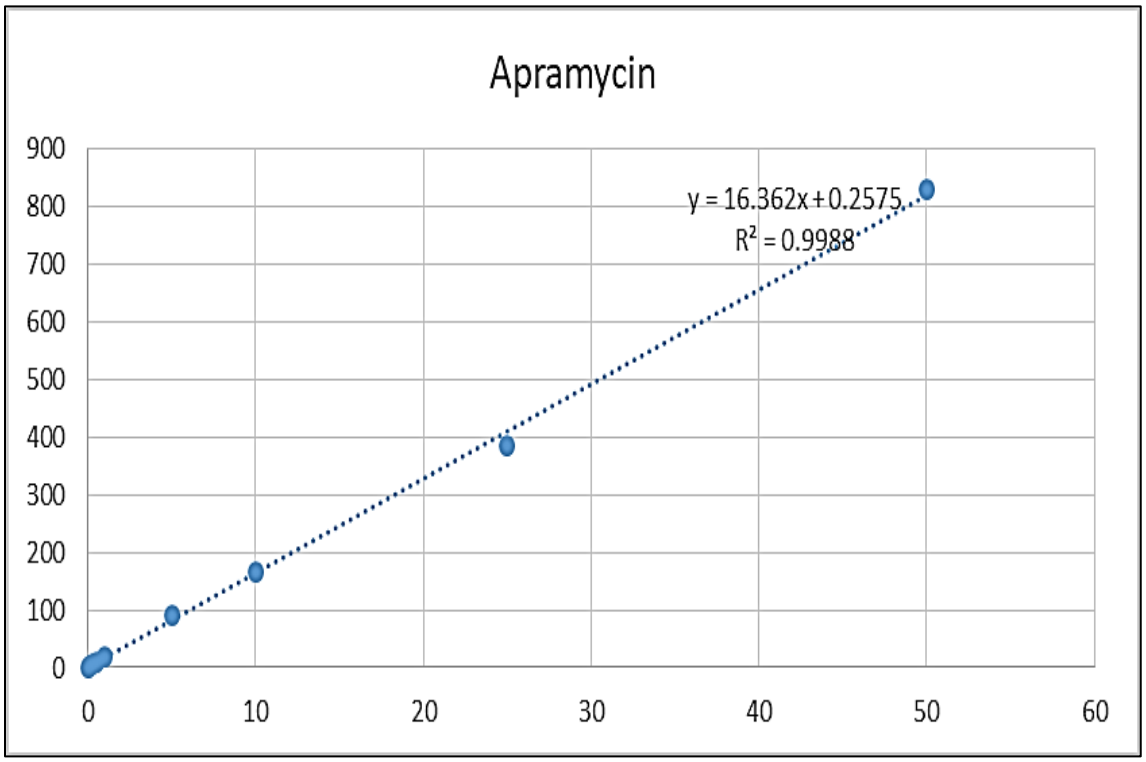

Figure 1. Calibration curve validation of apramycin in normal chickens serum (n=9).

Table 1. Serum concentrations of apramycin (µg/ml) in infected broiler chickens after a single IV injection of 10 mg/kg b.wt. of apramycin (n=6).& after oral intake of 25 mg/kg b.wt. of apramycin with single dose (µg/ml) (n=6).

Apramycin serum concentration (µg/ml)			
IV apra		**P.O apra**	
Time	**I.V apra ±S.E.**	**Time**	**P.O apra ±S.E.**
0.08 h	19.288± 0.322	**0.25h**	0.173± 0.010
0.17 h	16.017± 0.158	**0.33h**	0.218 ± 0.011
0.25 h	13.102± 0.209	**0.5h**	0.517± 0.023
0.5 h	9.543± 0.232	**1h**	1.165 ±0.071
1 h	7.045± 0.303	**2h**	3.235 ±0.078
2 h	5.493± 0.225	**4h**	2.433 ± 0.126
4 h	3.525± 0.145	**6h**	1.085±0.082
8h	2.410± 0.127	**8h**	0.633±0.021
12h	0.900± 0.065	**12h**	----------
24h	0.200± 0.015	----------	----------

Table 2. kinetic parameters of apramycin in infected birds after a single IV injection of 10 mg/kg b.wt. (n=6) & after oral administration of 25 mg/kg b.wt. of apramycin with single dose (µg/ml) (n=6).

Parameter	**Units**	**IV apra, ±SE**	**P.O apra , ±S.E**
˚Cp	**µg/ml^{-1}**	23.705 ±0.436	……
A	**µg/ml**	16.01± 0.65	……
α (Kab)	**h^{-1}**	3.93± 0.29	0.99 ± 0.16
t0.5 (α)	**h**	0.18± 0.01	0.81± 0.14
C_{max}	**µg/ml^{-1}**	……	3.273 ± 0.10
T_{max}	**h**	……	2.29± 0.06
B	**µg/ml**	7.69± 0.48	……
β	**h^{-1}**	0.17± 0.01	……
t0.5 (β)	**h**	4.27± 0.29	1.65± 0.11
K12	**h^{-1}**	2.22± 0.16	……
K21	**h^{-1}**	1.40± 0.16	……

K_{el}	**h^{-1}**	0.47± 0.02	0.43 ± 0.03
AUC	**µg/ml.h**	50.65± 1.40	15.25± 0.63
AUMC	**µg/ml.h^{-2}**	288.86±24.96	……
Cl_{tot}	**L/Kg/h**	0.10± 0.01	1.653± 0.066
MRT	**H**	5.66± 0.36	……
Vss	**L/kg**	1.11± 0.05	……
Vd_{area}	**L/kg**	0.423 ±0.008	……
F	**%**	……	12.04±0.4

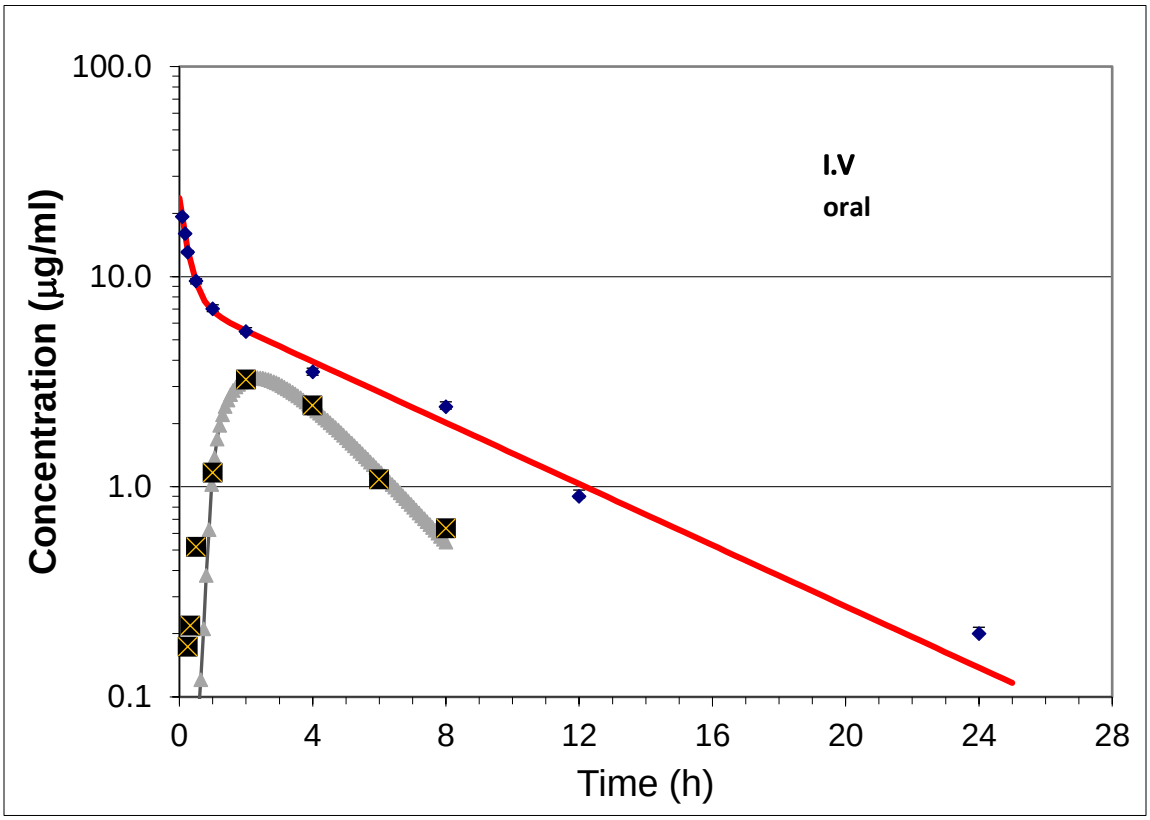

Figure 2. Semi-logarithmic chart presenting serum time-concentration of apramycin in infected broiler chickens' sera following single IV (10mg/kg b.wt.) and oral intake (25 mg/kg b.wt.) of apramycin (n = 6).

Table 3. Serum concentrations of apramycin (µg/ml) in infected broilers after a single IV injection of 10 mg/kg b.wt. of apramycin and IV injection 10 mg/kg b.wt. of cefotaxime chickens & after an oral administration of 25 mg/kg b.wt. of apramycin and intramuscular injection 10 mg/kg b.wt. of cefotaxime (n=6).

Apramycin concentrations (µg/ml serum)			
IV apra, IVcefo (infect)		**P.O apra , IM cefo (infect)**	
Time	**I.V ±S.E.**	**Time**	**P.O ±S.E.**
0.08 h	17.192± 0.082	**0.25h**	0.148± 0.006
0.17 h	13.902± 0.213	**0.33h**	0.182± 0.006
0.25 h	8.868± 0.371	**0.5h**	0.390± 0.024
0.5 h	6.218± 0.258	**1h**	1.108 ±0.028
1 h	3.517± 0.178	**2h**	2.297 ±0.014
2 h	1.965± 0.230	**4h**	1.972 ± 0.040
4 h	0.833± 0.032	**6h**	1.128±0.030
8h	0.170± 0.014	**8h**	1.030 ±0.021
12h	0.040± 0.009	**12h**	0.448 ± 0.032
24h	0.307± 0.016	----------	----------

Table 4. Pharmacokinetic parameters of apramycin (µg/ml) in normal chickens after a single IV injection of 10 mg/kg b.wt. of apramycin and IIV injection10 mg/kg b.wt. of cefotaxime & after an oral administration of 25 mg/kg b.wt. of apramycin and intramuscular injection 10 mg/kg b.wt. of cefotaxime (n=6).

Parameter	**Units**	**IV apra, IVcefo ±SE (Infect)**	**P.O apra , IM cefo ±S.E (infect)**
˚Cp	**µg/ml^{-1}**	23.774 ± 0.396	……
A	**µg/ml**	18.27± 0.48	……
α	**h^{-1}**	5.08± 0.27	0.488 ± 0.003
t0.5 (α)	**h**	0.14± 0.01	1.421± 0.009
C_{max}	**µg/ml^{-1}**	……	2.277 ± 0.025
T_{max}	**h**	……	2.456± 0.005
B	**µg/ml**	5.50± 0.28	……
β	**h^{-1}**	0.48± 0.03	……
t0.5 (β)	**h**	1.46± 0.07	1.414± 0.009

K12	**h^{-1}**	2.42± 0.15	……
K21	**h^{-1}**	1.55± 0.09	……
K_{el}	**h^{-1}**	1.59± 0.11	0.490 ± 0.003
AUC	**µg/ml.h**	15.20± 0.85	12.661± 0.200
AUMC	**µg/ml.h^{-2}**	25.33±2.69	……
Cl_{tot}	**L/Kg/h**	0.33± 0.03	……
MRT	**H**	1.64± 0.09	……
Vss	**L/kg**	1.08± 0.02	……
Vd_{area}	**L/kg**	0.421 ±0.007	……
F	**%**	……	33.31±0.325

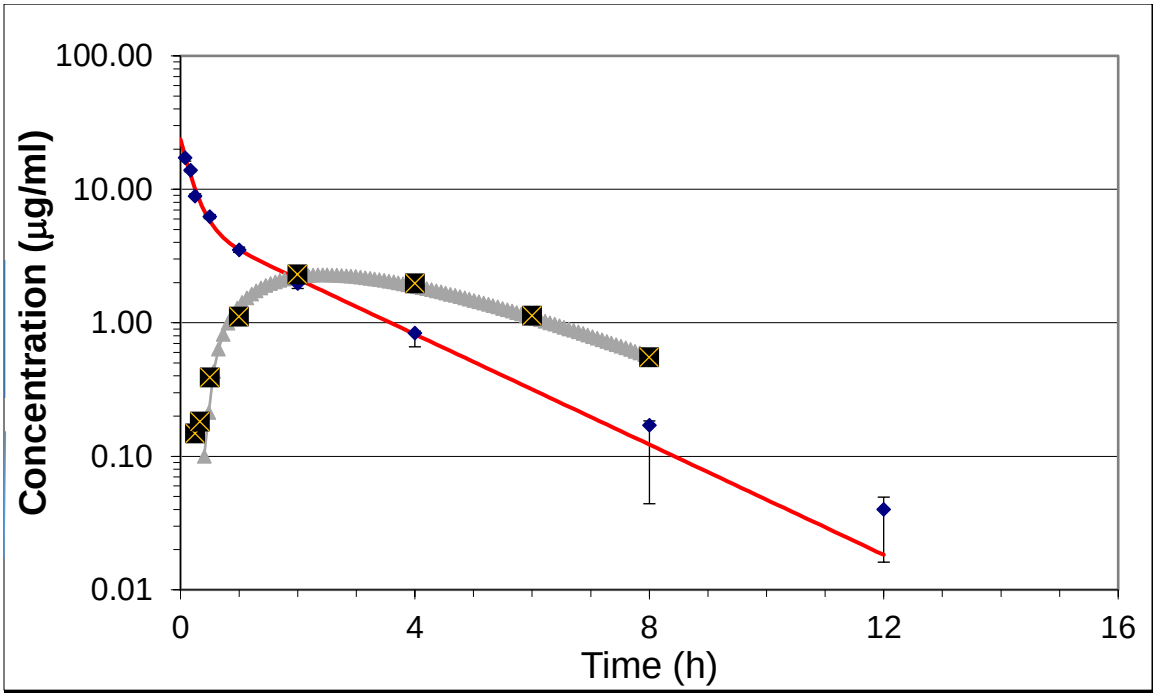

Figure 3. Semi-logarithmic chart presenting serum time-concentration of apramycin in infected chickens' sera after a single IV injection of 10 mg/kg b.wt. of apramycin and IV injection 10 mg/kg b.wt. of cefotaxime and oral intake of 25 mg/kg b.wt. of apramycin and intramuscular injection 10 mg/kg b.wt. of cefotaxime (n = 6).

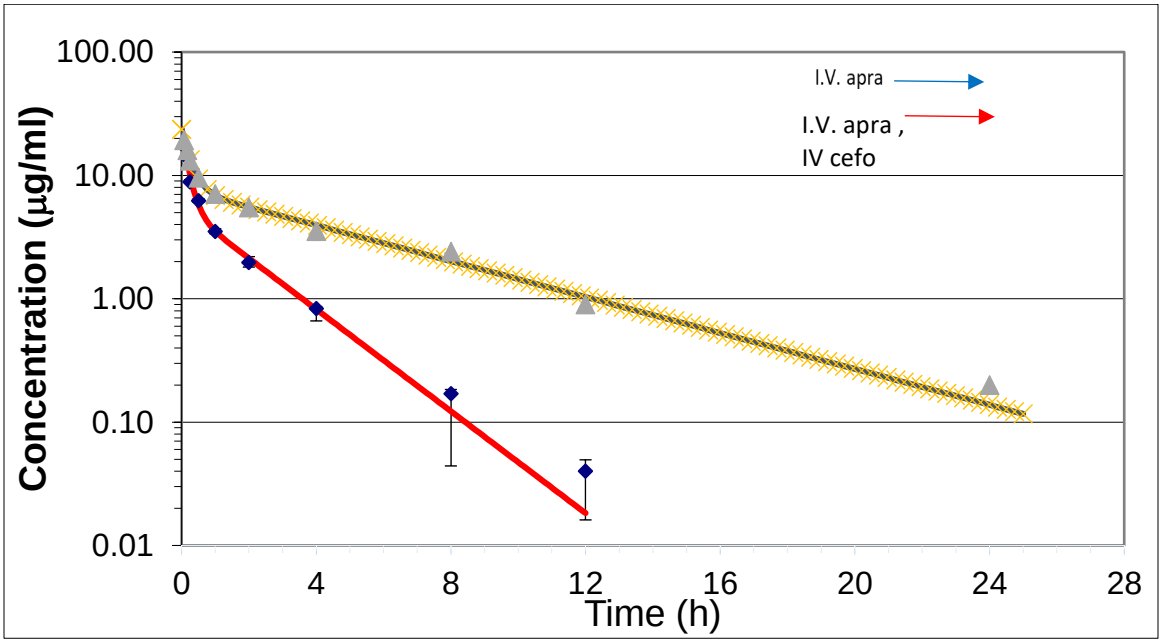

Figure 4. Semi-logarithmic chart presenting serum time-concentration of apramycin in infected chickens' sera after a single IV injection of 10 mg/kg b.wt. of apramycin and IV injection of 10 mg/kg b.wt. of apramycin concurrent with IV injection 10 mg/kg b.wt. of cefotaxime (n = 6).

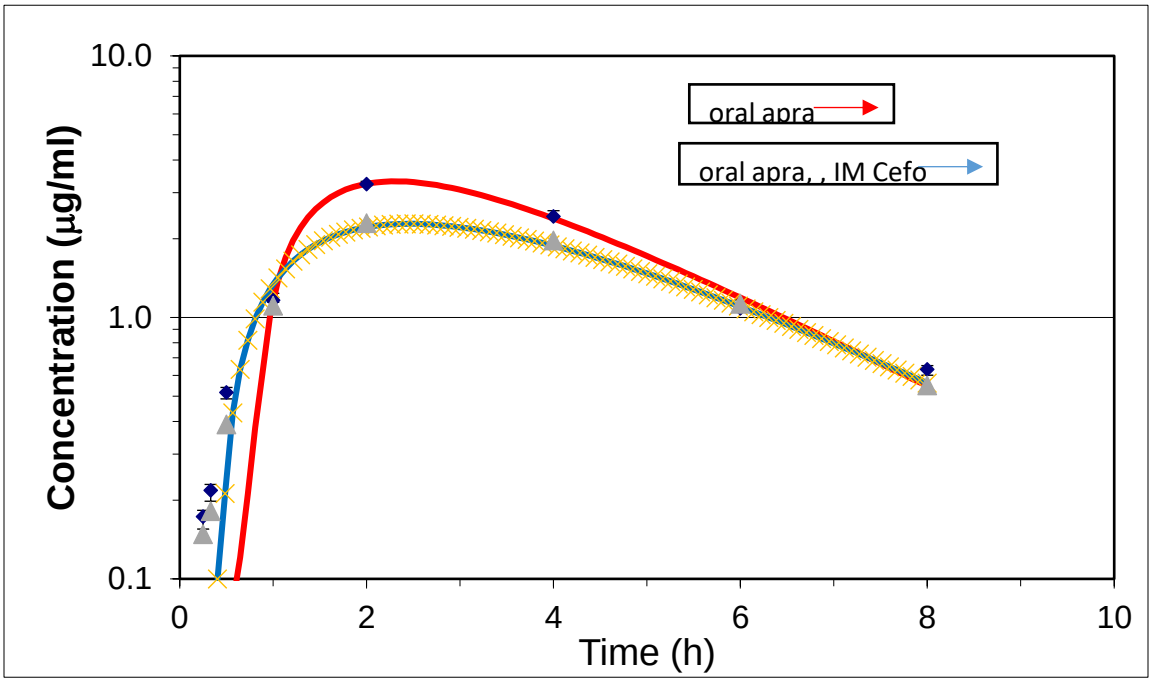

Figure 5. Semi-logarithmic chart presenting serum time-concentration of apramycin in infected chickens' sera after a single oral administration of 25 mg/kg b.wt. of apramycin and oral intake of 25 mg/kg b.wt. of apramycin concurrent with IM injection 10 mg/kg b.wt. of cefotaxime. (n = 6).

4. DISCUSSION

After IV injection, the serum concentration and pharmacokinetic characteristics of apramycin in broilers infected with E. coli

Chicken was experimentally infected with *E.Coli* O78. *E.Coli* O78 was obtained from serology unit in Animal Health Research Institute. Two days post infection, appearance signs of depression, off food, bloody diarrhea and difficult breathing with severe PM 1eslons (air saculitis, pericarditis and per hepatitis) and high mortality rate 30 %). Six infected chicken was IV injected with therapeutic apramycin 10 mg/kg b.wt. with a single dose.

Following a single IV injection in infected broiler chicken, Apramycin was rapidly distributed with distribution phase {α} equal to 3.93± 0.29h^{-1} and with short half –life as indicated by the value of t1/2α of (0.18± 0.01h). This value compared with values recorded in administrating IV apramycin only t1/2α of 0.24 ± 0.04h showed that slightly low in distribution half-life. Shorter half-life of distribution was recorded for gentamicin in febrile goats after single IV dose t1/2α (0.05h) (Ahmad *et al*., 1994), gentamycin against Pasteurella haemolytica t1/2α 0.23h (Burrows *et al*., 1986). Lengthier half-life of distribution was documented for gentamicin in bronchopneumonic calves t1/2α 3.06 h (Hunter *et al*., 1991).

The half-life of elimination (t0.5β) of 4.27± 0.29 hours. This value compared with values recorded in administrating IV apramycin only (t0.5β 5.45 ± 0.47) showed that slighty low in elimination half-life. These value is higher than gentamycin against Pasteurella haemolytica t1/2α 2.07h (Burrows *et al*., 1986), gentamicin in cats taken *E-coli* endotoxin 1.08 h (Jernigan *et al*., 1988), gentamycin in endotoxin-treated rats 1.17 + 0.15 h (Tardif *et al*., 1990), gentamicin in bronchopneumonic calves (t0.5β) 0.48 h (Hunter *et al*., 1991), gentamicin in febrile goats (t0.5β) 2.26 h (Ahmad *et al*., 1994). Similar to those reported in gentamicin against pseudomonas aeruginosa infected sheep 3.23 ±0.59 (lashev *et al*., 2001).

The total clearance of drug (CL_{tot}) was 0.10± 0.01L/kg/h. This value compared with values recorded in administrating IV apramycin only (CL_{tot} 0.083 ± 0.003 L/kg/hr) indicated slightly similar in the total body clearance. This obtained value closely similar to that previously reported of gentamycin against Pasteurella haemolytica (0.145) (Burrows *et al*., 1986), gentamicin in bronchopneumonic calves 5.96 L/kg/h (Hunter *et al*., 1991), gentamicin against pseudomonas aeruginosa infected sheep (0.082±0.012) (lashev *et al*., 2001). This rate was lesser than gentamicin in febrile goats 1.6 L/kg/h (Ahmad *et al*., 1994).

In the current study the apparent volume of distribution of apramycin in infected chicken after IV injection was 0.423 ± 0.008L/kg where volume of distribution steady – state {V_{dss}} was 1.11 ± 0.05L/kg. This observation was higher than apramycin administrated only {V_{dss}} was 0.970 ± 0.049 L/kg. This obtained value closely similar to that previously reported of gentamycin against Pasteurella haemolytica 0.434 L/kg (Burrows *et al*., 1986), gentamycin in endotoxin-treated rats 0.432 L/kg (Tardif *et al*., 1990), gentamicin in febrile goats 0.235 L/kg (Ahmad *et al*., 1994), gentamicin against pseudomonas

aeruginosa infected sheep Vd_{area} 0.363±0.055 L/kg (lashev *et al*., 2001). This value was lower than gentamicin in bronchopneumonic calves Vd_{area} 3.29 L/kg, {V_{dss}} 0.859 L/kg (Hunter *et al*., 1991).

Following single IV administration of 10 mg/kg b.wt of apramycin in infected chicken , apramycin was moved from central to peripheral compartment at a faster rate (K12 = 2.22± $0.16h^{-1}$) than its path from peripheral compartment to central compartment (K21 =1.40± $0.16h^{-1}$). This value was higher than gentamycin against Pasteurella haemolytica (K12 = 0.026 min^{-1}) (K21 = 0.0212 min^{-1}) (Burrows *et al*., 1986), gentamicin in febrile goats (K12 = 0.143 min^{-1}) (K21 = 0.056 min^{-1}) (Ahmad *et al*., 1994).

Serum concentration and kinetic parameters of apramycin in *E.coli* infected broilers after the oral administration

After 15 days the identical infected chicken that had a 10 mg/kg b.wt IV injection of apramycin, reinfected with *Escherichia coli* O78 one more time. Two days post infection, appearance signs of infection, the infected chicken taken 25 mg/kg b.wt of apramycin.

Following a single oral intake of apramycin 25mg/kg.b.wt.in infected chicken, the drug reached its maximum serum concentrations Cmax (3.273 ± 0.10 µg/ml) at Tmax (2.29± 0.06 hours) of administration. These values were similar to those recorded of apramycin in healthy group Cmax (3.255 ± 0.03µg/ml) achieved at (tmax) (2.59± 0.03hours). This value is higher than those stated of apramycin in experimentally *E.coli* infected chickens Cmax (0.765±0.018) at (tmax) (0.797±0.019) (El-Sayed *et al*., 2018). The obtained result differ from those recorded of apramycin against salmonella in pig Cmax was shorter (0.24 ± 0.01 µg/ml) at longer (tmax) 4.0 ± 0.0h (Dai *et al*., 2022). This value was higher than that seen for other species when administered by intramuscular route such as apramycin in milk in infected lactating ewe Cmax (0.71± 0.26 g/ml) at (tmax) 3.0 ± 0.0 h (Ziv *et al*., 1995).

In the recent study, apramycin was poor half-life of absorption ($T_{0.5\ (ab)}$) 0.81± 0.14h. These values were lower than those recorded of apramycin in the healthy group 1.51 ± 0.09h. The obtained result is higher than those reported of apramycin in experimentally *E. coli* infected chickens (T0.5 (ab)) 0.076±0.002h (El-Sayed *et al*., 2018).

The half-life of elimination {$t_{0.5\ (\beta)}$} of apramycin was1.65± 0.11h which lower to those reported for of apramycin in healthy group (1.93± 0.15 h). The obtained result is higher than those reported of apramycin in experimentally *E.coli* infected chickens (T0.5 (β)) 0.553±0.013 h (El-Sayed *et al*., 2018) and lower than apramycin administrated against salmonella in pig15.77± 1.93h (Dai *et al*., 2022).This value was higher than that seen for other species when administered by intramuscular route such as apramycin in milk in infected lactating ewe (T0.5 (β)) 2.51h (Ziv *et al*., 1995).

The considered AUC was set up to be 15.25± 0.63µg/h/ml which lower than values recorded in administrating oral apramycin only21.81± 0.52 µg/h/ml.

The result is higher than those reported of apramycin in experimentally *E.coli* infected chickens 1.66±0.040 µg/h/ml (El-Sayed *et al*., 2018), and higher than

apramycin administrated against salmonella in pig 0.24± 0.01 µg/h/ml. (Dai *et al*., 2022), This value was higher than that seen for other species when administered by intramuscular route such as apramycin in milk in infected lactating ewe 45.4 ± 4.3 (µg/min/mL) (Ziv *et al*., 1995).

Following oral management, the systemic bioavailability of apramycin following its oral single dose of 25 mg/kg b. wt. in infected birds was 12.04±0.4%.This value indicate that apramycin is not well absorbed from intestine after infection with *E.coli*. This value is similar to value recorded in administrating oral apramycin in healthy group (11.60±1.2%).

Effect of cefotaxime-apramycin combination on the serum concentration and kinetic parameters of apramycin in *E.coli* infected broilers.

Six infected broiler chicken administrated IV injection of 10 mg/kg b.wt of apramycin and IV injection 10 mg/kg b.wt. of cefotaxime.

In infected with *E.coli* ,apramycin was distributed{α} equal to 5.08± 0.27 h^{-1} in the body with a half-life of distribution ($t_{0.5\alpha}$) of 0.14± 0.01h , this value compared with values recorded in administrating IV apramycin , IV cefotaxime in healthy group (t0.5α) of 0.12 ± 0.30 h showed that similar in distribution half-life.

The elimination half-life (t0.5β) of 1.46± 0.07 hours, this value compared with values recorded in administrating IV apramycin with IV cefotaxime in healthy group {t0.5(β)} value of 4.45± 0.22 h showed that decrease in elimination half-life.

The total clearance (CL_{tot}) was 0.67± 0.04L/kg/h, this value compared with values recorded in administrating IV apramycin with IV cefotaxime in healthy group (CL_{tot}) (0.110± 0.009 L/kg/hr) showed that relatively increasing in the total body clearance.

In the current study the apparent volume of distribution was 0.421 ±0.007 L/kg where volume of distribution at steady – state {Vdss} was 1.08± 0.02 L/kg, this value compared with values recorded in administrating IV apramycin with IV cefotaxime in healthy group {Vdss} was 1.05± 0.01 L/kg showed that relatively similar volume of distribution at steady – state

Apramycin was moved from central to peripheral compartment at a faster rate (K12 = 2.42± 0.15 h-1) than its passage from peripheral compartment to central compartment (K21 =1.55± 0.09h-1). This value compared with values recorded in administrating IV apramycin with IV cefotaxime in healthy group (K12 = 3.52± 0.22h-1) (K21 =1.78± 0.08 h-1) showed that the lower the distribution of drug into the peripheral compartment.

Effect of cefotaxime on the serum concentration and pharmacokinetic parameters of apramycin infected broilers following oral administration of 25 mg/kg b.wt of apramycin and IM injection 10 mg/kg b.wt of cefotaxime.

After 15 days the same infected chicken which administrated IV injection of 10 mg/kg b.wt of apramycin and IV injection 10 mg/kg b.wt of cefotaxime, reinfected with *E coli* O78 one more time. Two days post infection, appearance signs of infection, the infected chicken taken 25 mg/kg b.wt of apramycin and intramuscular injection 10 mg/kg b.wt of cefotaxime.

Following oral administration of 25 mg/kg. b.wt of apramycin and intramuscular injection 10 mg/kg b.wt of cefotaxime in infected chicken, the drug got its maximum serum concentrations Cmax (2.277± 0.025 µg/ml) at T_{max} (2.456 ± 0.005 hours) of administration. This value lower than recorded in administrating oral apramycin and intramuscular injection of cefotaxime in healthy chicken C_{max} (2.505 ± 0.032 µg/ml) at T_{max} (2.602± 0.007hours). On the other hand these values were lower than that seen for other species when administered by methods other than the oral route such as in Kanamycin and ampicillin intramuscular injection after induction of *E coli* lipopolysaccharide,) C_{max} (10.6 ± 1.6) T_{max} (0.8 ± 0.3 hours) (Firth *et al*., 1988), amikacin and imipenem against *Acinetobacter baumannii pneumonia* C_{max} (21.6 µg/ml) (Bernabeu *et al*., 2005).

The absorption rate constant {Kab} of 0.488 ± 0.003 h^{-1}, while absorption half-life {t0.5 (ab)} was 1.421± 0.009 h. This value similar that recorded in administrating oral apramycin and intramuscular injection of cefotaxime in healthy chicken {t0.5 (ab)} 1.309± 0.085h. On the other hand these values were lower than that seen for other species when administered by methods other than the oral route such as in Kanamycin and ampicillin intramuscular injection after induction of *E coli* lipopolysaccharide (0.12 ± 0.04h) (Firth *et al*., 1988).

Following oral administration of 25 mg/kg. b.wt. of apramycin and intramuscular injection 10 mg/kg. b.wt of cefotaxime in infected chicken ,apramycin was eliminated at rate {Kel} equal to 0.490 ± 0.003 h-1 and the elimination half-life {t0.5 (β)} was 1.414 ± 0.009 h. comparing with that recorded in administrating oral apramycin and intramuscular injection of cefotaxime in healthy chicken eliminated at rate {Kel} equal to 0.307 ± 0.025 h-1 and the elimination half-life {t0.5 (β)} was 2.328 ± 0.174h show that decrease in elimination half-life. On the other hand these values were lower than that seen for other species when administered by methods other than the oral route such as in Kanamycin and ampicillin intramuscular injection after induction of *E coli* lipopolysaccharide (1.43 ± 0.31h) (Firth *et al*., 1988) show similarity in elimination half-life. Amikacin and imipenem against *Acinetobacter baumannii pneumonia* (0.9 h) (Bernabeu *et al.*, 2005).

The considered AUC was established to be 12.661 ± 0.200 µg/h/ml. which decrease comparing with that recorded in administrating oral apramycin and intramuscular injection of cefotaxime in healthy chicken17.550± 0.361µg/h/ml. These values were lower than those recorded Kanamycin and ampicillin intramuscular injection after induction of *E coli* lipopolysaccharide (28.5 ± 6.13 µg/h/ml) (Firth *et al*., 1988). Amikacin and imipenem agaist *Acinetobacter baumannii pneumonia* 27.2 µg/h/ml (Bernabeu *et al*., 2005).

Following oral administration of 25 mg/kg b.wt of apramycin and intramuscular injection of 10 mg/kg. b.wt of cefotaxime, the calculated (F%) bioavailability of apramycin in infected birds was 33.31±0.32%.

CONCLUSION

Apramycin's oral bioavailability was low 12.04%, indicating poor oral absorption; hence, it is advised to use it to treat enteric infectious disorders caused by *E.*

coli and *Salmonella* species. The conclusion is that apramycin exhibits pharmacokinetic behavior similar to aminoglycoside antibiotics following IV and oral dosing to broiler chickens. The experiment's above results made it abundantly evident that cefotaxime affected the rate at which apramycin was distributed, and that when cefotaxime was administered with apramycin, the latter was distributed more quickly in bodily fluids and tissues. It is advised to use it to treat enteric infectious disorders caused by E. *coli* and *Salmonella* species as apramycin's oral bioavailability was low 12.02% indicating poor oral absorption from intestine which improved when given concurrent with cefotaxime with bioavailability equal to 33.31%.

REFERENCES

Ahmad, A. H., Bahga, H. S., & SHARMA, L. (1994). Pharmacokinetics of gentamicin following single dose intravenous administration in normal and febrile goats. Journal of veterinary pharmacology and therapeutics, 17(5), 369-373.

Baxla, S. L., Kumar, M., Jayachandran, C., Roy, B. K., & Kumari, A. (2010). Pharmacokinetics of Gentamicin and its Interaction with Paracetamol after IV administration in Buffalo Calves (Bubalus bubalis). Journal of Bioanalysis & Biomedicine, 2(3), 065-068.

Bernabeu-Wittel, M., Pichardo, C., Garcia-Curiel, A., Pachón-Ibáñez, M. E., Ibáñez-Martínez, J., Jiménez-Mejías, M. E., & Pachon, J. (2005). Pharmacokinetic/pharmacodynamic assessment of the in-vivo efficacy of imipenem alone or in combination with amikacin for the treatment of experimental multiresistant Acinetobacter baumannii pneumonia. Clinical microbiology and infection, 11(4), 319-325.

Burrows, G. E., Barto, P. B., & Martin, B. (1986). Antibiotic disposition in experimental pneumonic pasteurellosis: gentamicin and tylosin. Canadian Journal of Veterinary Research, 50(2), 193.

Dai, C., Zhao, T., Yang, X., Xiao, X., Velkov, T., & Tang, S. (2017). Pharmacokinetics and relative bioavailability of an oral amoxicillin-apramycin combination in pigs. PLoS One, 12(4), e0176149.

Dai, X., Gu, Y., Guo, J., Huang, L., Cheng, G., Peng, D., & Hao, H. (2022). Clinical Breakpoint of Apramycin to Swine Salmonell a and Its Effect on Ileum Flora. International Journal of Molecular Sciences, 23(3), 1424.

Elbadawy, M., & Aboubakr, M. (2017). Pharmacokinetic, bioavailability and tissue residues of apramycin in broiler chickens. International Journal of Pharma Sciences, 7(4), 1826-1831.

EL. Sayed, M.G.A. EL-Komy, A.A.A. and Soliman, A. Asmaa (2018). pharmacokinetics, Bioavailability and tissue residues of apramycin in normal chickens and *Escherichia coli* infected broiler chickens. World Journal of pharmacy and pharmaceutical Sciences, 7(4), 194-206

Firth, E. C., Klein, W. R., Nouws, J. F. M., & Wensing, T. (1988). Effect of induced synovial inflammation on pharmacokinetics and synovial concentration of sodium ampicillin

and kanamycin sulfate after systemic administration in ponies. Journal of veterinary pharmacology and therapeutics, 11(1), 56-62.

Hesham, S. T., & Sultan, H. M. (2019) Effect of dose escalation and route of administration on tissue residues profile and withdrawal times of gentamicin and apramycin in broiler chickens. Animal Health Research Journal, 7(2): 147-162.

Hunter, R. P., BROWN, S. A., Rollins, J. K., & Nelligan, D. F. (1991). The effects of experimentally induced bronchopneumonia on the pharmacokinetics and tissue depletion of gentamicin in healthy and pneumonic calves. Journal of veterinary pharmacology and therapeutics, 14(3), 276-292.

Jernigan, A. D., Hatch, R. C., Wilson, R. C., Brown, J., & Tuler, S. M. (1988). Pharmacokinetics of gentamicin in cats given Escherichia coli endotoxin. American Journal of Veterinary Research, 49(5), 603-607.

Kotra, L. P., Haddad, J., & Mobashery, S. (2000). Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. Antimicrobial agents and chemotherapy, 44(12), 3249-3256.

Lashev, L. (1998). Pharmacokinetics of apramycin and sulphachlorpyridazine coadministered with flavophospholipol and avoparcin to chickens. Bulg J Vet Med, 1, 71-76.

Lashev, L., & Lasarova, S. (2001). Pharmacokinetics and side-effects of gentamicin in healthy and pseudomonas aeruginosa infected sheep. Journal of Veterinary Pharmacology and Therapeutics, 24(3), 237-240.

Lashev, L. D., Pashov, D. A., & Marinkov, T. N. (1992). Interspecies differences in the pharmacokinetics of kanamycin and apramycin. Veterinary research communications, 16(4), 293-300.

Pacifici, G.M. & Marchini, G. (2017). Clinical Pharmacology of Cefotaxime in Neonates and Infants: Effects and Pharmacokinetics. Int J Pediatr 2017; 5(11): 6111-38.

Riedel, S., Vijayakumar, D., Berg, G., Kang, A. D., Smith, K. P., & Kirby, J. E. (2019). Evaluation of apramycin against spectinomycin-resistant and-susceptible strains of Neisseria gonorrhoeae. Journal of Antimicrobial Chemotherapy, 74(5), 1311-1316.

Snedecor, G.W. (1969) Statistical methods. 4th ed. Ames, IA: The Lowa state University Press, 91.

Tardif, D. O. M. I. N. I. Q. U. E., Beauchamp, D. E. N. I. S., & Bergeron, M. G. (1990). Influence of endotoxin on the intracortical accumulation kinetics of gentamicin in rats. Antimicrobial agents and chemotherapy, 34(4), 576-580.

Tatsuta, K., Akimoto, K., Takahashi, H., Hamatsu, T., Annaka, M., & Kinoshita, M. (1984). Total synthesis of aminoglycoside antibiotics, apramycin and saccharocin (KA-5685). Bulletin of the Chemical Society of Japan, 57(2), 529-538.

Varghese, R., Basu, S., Neeravi, A., Pragasam, A., Aravind, V., Gupta, R., Miraclin, A., Ramaiah, S.,

Anbarasu, A. and Veeraraghavan, B. (2022). Emergence of Meropenem Resistance Among Cefotaxime Non-susceptible Streptococcus pneumoniae: Evidence and Challenges. Front. Microbiol. 12:810414.

Xu, Y.B., Ouyang, Y. & Zhao, D. (2020). Curative effects of vancomycin and cefotaxime combined with gamma globulin respectively in neonatal septicemia and their influences on PCT, CRP and hs-CRP. European Review for Medical and Pharmacological Sciences. 24: 4486-4494.

Walton, J. R. (1978). Apramycin, a new aminocyclitol antibiotic: I. In vitro microbiological studies. Journal of Antimicrobial Chemotherapy, 4(4), 309-313.

Zhang, X. Y., Ding, L. J., & Fan, M. Z. (2009). Resistance patterns and detection of aac (3)-IV gene in apramycin-resistant Escherichia coli isolated from farm animals and farm workers in northeastern of China. Research in veterinary science, 87(3), 449-454.

Ziv, G., Kurtz, B., Risenberg, R., & Glickman, A. (1995). Serum and milk concentrations of apramycin in lactating cows, ewes and goats. Journal of Veterinary Pharmacology and Therapeutics, 18(5), 346-351.