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Pharmacokinetic, bioavailability and tissue residues of apramycin in broiler chickens

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ABSTRACT

The pharmacokinetics (after single oral, i.m. and i.v. administration) and tissue residues after repeated oral (daily for five days) and i.m. (daily for two days) administration of apramycin were investigated in healthy broiler chickens. Apramycin was administered at a dose level of 25 mg/kg b. wt. (for oral and i.m. administration) and at 10 mg/kg b. wt. for i.v. injection. Seventy clinically healthy Hubbard chickens of 1.65~2 kg b.wt. and of 45 days old, were used in the current study. The maximum plasma concentrations of apramycin were achieved 0.18 and 0.70 h after oral and i.m. administration with an absorption half-life ($t_{1/2ab}$) of 0.11 and 0.18 h and an elimination half-life ($t_{1/2\beta}$) of 1.23 and 2.33 h, respectively. The systemic bioavailability was 2.5 and 60.5 % after oral and i.m. administration, respectively, indicating poor oral absorption of apramycin. After i.v. injection, the pharmacokinetics of apramycin was best described by a two-compartment open model with a $t_{1/2\alpha}$ of 1.55 h, $t_{1/2\beta}$ of 2.15 h, V_{dss} of 4.85 litre/kg and Cl_{β} of 1.9 litre/kg/h. The plasma protein binding of apramycin was 25.0 ± 2.45 %. The highest tissue concentrations of apramycin were present in kidneys and liver. No apramycin residues were detected in tissues after 6 h except in liver and kidneys following oral dosing.

Keywords: Apramycin; Pharmacokinetics; Bioavailability; Residues; Broiler chickens.

1. INTRODUCTION

Apramycin is a bactericidal aminocyclitol broad-spectrum antibiotic primarily prescribed for medication of systemic and enteric infections caused by Gram-negative bacteria in a variety of animals species. It acts by irreversible binding to the 30S ribosomal subunit thereby inhibiting protein synthesis. It is active against many Gram-negative bacteria (*E. coli*, *Pseudomonas* spp., *Salmonella* spp., *Klebsiella* spp., *Proteus* spp., *Pasteurella* spp., *Treponema hyodysenteriae* and *Bordetella bronchiseptica*). In addition, it is also active against *Staphylococcus* spp. and *Mycoplasma* spp [1-5]. It is generally poorly absorbed from gastrointestinal tract of animals [6] and active *in vitro* against *Salmonella* spp. and *Escherichia coli* strains that are resistant to neomycin and dihydrostreptomycin [3-5]. Oral and parenteral preparations of apramycin are commercially available in many countries. The pharmacokinetic profile of apramycin has been extensively investigated in many animal species; in turkey [5], in calves [4, 7], in sheep, rabbits, chickens and pigeons [8], in Japanese quail [9], in lactating cows, ewes and goats, [4], in chickens [10-12], in turkeys roosters and hens [13]. The large-scale use of the orally administered aminocyclitols such as apramycin for treatment of enteric infectious diseases caused by *Salmonella* spp. and *E. coli* in poultry and the problems of their residues in meat necessitates further studies on the pharmacokinetics of apramycin in broiler chickens. To our knowledge, little information is available regarding its pharmacokinetics and tissue residues in broiler chickens. Therefore, the present study was conducted to study the pharmacokinetic and residues profile of apramycin (apracolin®) following oral, intramuscular and intravenous administration to broiler chickens.

2. MATERIALS AND METHODS

2.1 Drugs

Apramycin pure powder was kindly supplied as a pure powder from ATCO Pharma for Pharmaceutical

Industries, Egypt. This powder was used for i.m. and i.v. injection. Apramycin has the chemical name of D-Streptamine, 4-0- [(8R)-2-amino-8-0-(4-amino-4-deoxy-a-D-glucopyranosyl)-2,3,7-trideoxy-7-(methylamino)-D-glycero-a-D-allo-octodialdo-1,5: 8, 4-dipyranos-1-yl]-2-deoxy-sulphuric acid salt with molecular formula of $C_{21}H_{41}N_5O_{11}.5/2H_2SO_4$ and of molecular weight of 539.6 for its base and 784.8 g/mol for its salt .

Apracolin® W.S.P., (ATCO Pharma for Pharmaceutical Industries, Egypt) was used for oral administration. Each 100 gm contains 86.548 gm of apramycin sulphate (eq. to 59.524 gm Apramycin base).

2.2 Experimental chickens

Seventy clinically healthy Hubbard chickens with body weights of 1.65 to 2.0 kg and of 45 days old were used in the current study. Chickens were of both sexes and purchased from a local poultry farm. During 10 days acclimatisation and subsequent treatment periods, they had a free access to water and feed which was free from antibacterial drugs. The birds were housed in groups of five chickens per cage. The experiments were performed in accordance with the guidelines set by the Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt.

2.3 Experimental design

2.3.1 Pharmacokinetic study

Ten chickens of 45 days old weighing about 1.75 ~ 2.0 kg were used and placed into two groups, each of five birds. Ration was withheld 12 h before oral giving of drug and was offered 5 h after drug administration. Chickens of the first group was given a single oral (directly into the crop using gavage) dose of apramycin (25 mg/kg b. wt.) and those of the second group were administered the same dose of apramycin intramuscularly in thigh muscle. This dose was selected according to the manufacturer's approved daily dose, which falls within the range applied by some researchers on efficacy studies of apramycin on colonization of pathogenic *E. coli* in intestinal tract of broiler chicks [15, 16]. Blood samples (1-1.5 mL) were collected from left wing vein or other veins into heparinized tubes at 0 (before dosing), 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing to determine the drug concentration in plasma. The samples were centrifuged at 1000 ***g*** for 5 minutes and then plasma was collected and stored at -20°C until analysis. Two weeks later, apramycin was injected intravenously to the same chickens at a dose level of 10 mg/kg b. w.t. to study bioavailability of apramycin. The plasma protein binding percentage of apramycin was determined *in vitro*.

The pharmacokinetic parameters of apramycin in chickens were calculated according to the method of Baggot, (1978b) [16]. The following parameters were calculated: distribution and elimination phases (α, β) and their half-lives ($t_{1/2}\alpha$, $t_{1/2}\beta$) after i.v. injection, absorption rate constant (K_{ab}), absorption half-lives ($t_{1/2abs}$) after oral and i.m. administration, maximum plasma concentration after oral and i.m. administration (C_{max}), and time to reach this maximum (t_{max}). elimination rate (k_{el}), was determined by least-squares regression analysis of terminal log-linear phase of the plasma concentration-time profile (k_{el} = 2.303× slope), elimination half-life ($t_{1/2\beta}$) = 0.639/k_{el}; volume of distribution (V_{darea}), where V_{darea} = (dose/AUC)×β; volume of distribution at steady state (V_{dss}) was calculated as (Dose×MRT_{iv}/AUV_{iv}); total body clearance (CL_{tot}), where CL_{tot} = dose/AUC; area under plasma concentration-time curve (AUC) using linear trapezoid method with extrapolation to infinity; oral and i.m. bioavailability (F %) was calculated from the ratio between the value of AUC oral and/or i.m. for each individual chicken and the value of AUC i.v. for the same individual chicken as follow;

$$F\,(\%) = \frac{\text{AUC (oral or i.m for each chicken)} \times \text{Dose (i.v.)}}{\text{AUC (i.v for each chicken)} \times \text{Dose (oral or i.m.)}} \times 100$$

2.3.2 Tissue residues study

Sixty birds were placed in a two equal groups, each of 30 chickens. Birds of both groups was given apramycin (25 mg/kg b. wt.). Chickens of first group were given apramycin orally (via intra-crop) daily for five successive days while those of second group was injected the drug intramuscularly daily for two successive days. Five chickens were slaughtered at 0.25, 1, 3, 6, 12 and 24 h after the last administered oral dose and at 1, 2, 4, 5, 6 and 7 days after the last administered i.m. dose. Tissue samples of liver, kidney, lung, intestines, brain and breast muscle were taken from the slaughtered chickens. Two grams of each tissue was minced in test tube with 2 mL distilled water. Mixtures were homogenized in homogenizer, centrifuged at 1000 ***g*** for 5 minutes, and the supernatant fluid of each sample was collected and directly assayed microbiologically for assay of apramycin.

2.3.3 Analytical method

Apramycin in both plasma and tissue samples was determined by the microbiological assay method described by Bennett et al., (1966) [17] using Bacillus subtilis (ATCC 6633) as a test organism and apramycin standard solutions in plasma as reference concentrations. The present study revealed that the limit of quantification of apramycin in both plasma and tissues were 0.02 µg/mL and 0.05 µg/mL, respectively.

2.3.4 Plasma protein binding

The plasma protein binding of apramycin was determined *in vitro* [18]. The percentage of protein-bound fraction was calculated as follows:

$$\text{Protein binding \%} = \frac{\text{Concentration of apramycin bound to plasma proteins}}{\text{Concentration of apramycin in standard solution}} \times 100$$

Table 1: Plasma concentrations of apramycin (µg/mL) in chickens after a single oral and i.m. administration of 25 mg/kg b. w.t. and after a single i.v. injection of 10 mg/kg b. w.t.

Time (h)	Concentration (µg)		
	Oral (n = 5)	i.m. (n = 5)	i.v. (n = 10)
0.17	0.26±0.01	1.0±0.011	17.0±1.05
0.25	0.75±0.05	3.6±0.03	15.0±1.02
0.5	0.6±0.03	6.0±0.04	10.4±0.50
1	0.33±0.01	10.0±0.5	9.0±0.55
2	0.24±0.05	5.5±0.04	6.0±0.45
3	0.21±0.02	4.0±0.03	3.0±0.15
4	0.16±0.01	2.85±0.2	2.5±0.18
5	0.12±0.01	2.3±0.021	2.0±0.15
6	-	1.80±0.02	1.12±0.08
8	-	1.4±0.09	0.8±0.06
12	-	0.5±0.03	0.2±0.01

- = not detectable, the results are the mean ± SEM.

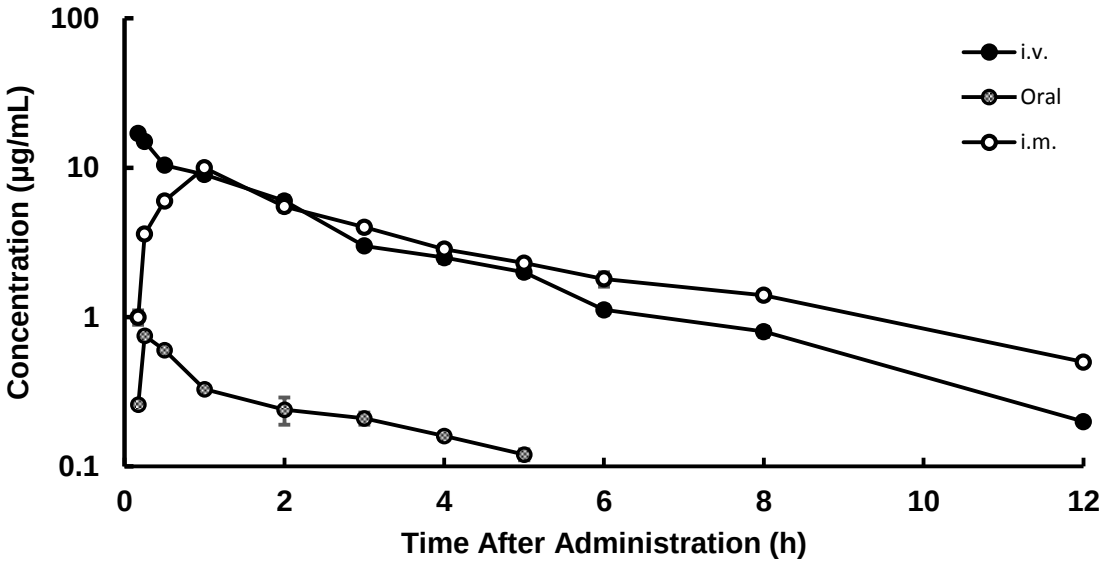

Figure 1. Semilogarthimic plot showing the plasma concentration-time curve of apramycin after its single oral, i.m. (25 mg/kg b. w.t.) and i.v. (10 mg/kg b. w.t.) administration to chickens. Each point and vertical bar represents the mean and standard error, respectively.

3. RESULTS AND DISCUSSION

3.1 Pharmacokinetics

Apracolin® was well tolerated by birds and there were no unexpected incidents that could have influenced the outcome of the study. The concentrations of apramycin in chicken plasma were determined for 24 h after the three administration. The mean concentration-time profiles of apramycin after a single oral, i.m. and i.v. administration of 25 mg/kg b.w.t. are shown in table 1 and fig. 1. The pharmacokinetic parameters are recorded in table 2. After i.v. administration, apramycin concentration revealed a bi-exponential decline that can be best fit by a two-compartment open model. Elimination half-life was 2.15±0.01h. The drug was rapidly distributed and the apparent volume of distribution was large (more than one litre/kg). After oral and i.m. administration, the absorption half-lives were 0.11±0.001 and 0.18±0.002 h, respectively and their corresponding t_{max} were 0.18±0.011 and 0.70±0.04 h, respectively indicating rapid absorption of apramycin. The bioavailability of apramycin after oral giving was very low (2.50±0.02%) while after i.m. injection was relatively high (60.5±2.85%). Other pharmacokinetic parameters were also determined and summarized in table 2. The plasma protein binding percentage of apramycin was 25.0 ± 2.45 %.

Table 2: Pharmacokinetic parameters of apramycin in broiler chickens after a single oral and i.m. administration of 25 mg/kg b. w.t. and after a single i.v. injection of 10 mg/kg b. w.t.

Parameter	Unit	Oral	i.m.	i.v.
		(*n* = 5)	(*n* = 5)	(*n* = 10)
α	h^{-1}	-	-	0.43±0.02
$t_{1/2\alpha}$	h	-	-	1.55±0.21
K_{ab}	h^{-1}	6.60±0.05	3.7±0.06	-
$t_{1/2abs}$	h	0.11±0.001	0.18±0.002	-
t_{max}	h	0.18±0.011	0.70±0.04	-
C_{max}	µg/ml	0.75±0.03	10.6±0.42	-
K_{el}	h^{-1}	1.22±0.02	0.55±0.003	0.45±0.003
β	h^{-1}	-	-	0.35±0.02
$t_{1/2\beta}$	h	1.22±0.01	2.31±0.02	2.15±0.01
$V_{d\ area}$	l/kg	-	-	5.55±0.13
V_{dss}	l/kg	-	-	4.85±0.07
CL_{β}	l/kg/h	-	-	1.9±0.05
AUC	µg/ml/h	0.83±0.02	25.2±1.07	40.1±1.02
F	%	2.50±0.02	60.5±2.85	-

- = not detectable, the results are the mean ± SEM.

Table 3: Plasma and tissue concentrations of apramycin (µg/ml or µg/g) after a multiple intracrop dosing of 25 mg/kg b. w.t. for five successive days in chickens (*n* = 5).

Tissue	Time of slaughter after the last dose (h)					
	0.25	1	3	6	12	24
Liver	0.31±0.01	0.14±0.011	0.09±0,002	0.05±0.001	-	-
Kidney	1.32±0.11	1.07±0.021	0.72±0.011	0.48±0.003	-	-
Lung	0.27±0.01	0.14±0.011	0.09±0.001	-	-	-
intestines	0.19±0.02	0.11±0.011	0.075±0.01	-	-	-
Brain	0.10±0.003	0.09±0.002	0.06±0.001	-	-	-
Breast muscle	0.14±0.0004	0.10 ±0.003	0.07±0.003	-	-	-

- = not detectable, the results are the mean ± SEM.

Table 4: Tissue concentrations of apramycin (µg/mL or µg/g) after a multiple i.m. dosing of 25 mg/kg b. w.t. for two successive days in chickens (*n* = 5)

Tissue	Time of slaughter after the last dose (day)					
	1	2	4	5	6	7
Liver	5.55±0.11	2.65±0.09	0.99±0.02	0.38±0.03	-	-
Kidney	16.0±1.4	9.55±0.37	4.23±0.15	1.55±0.08	0.85±0.02	-
Lung	4.75±0.09	1.95±0.07	0.85±0.04	0.28±0.01	-	-
intestines	0.89±0.01	0.65±0.03	0.35±0.02	-	-	-
Brain	1.59±0.02	0.74±0.02	0.35±0.01	-	-	-
Breast muscle	2.28±0.06	0.94±0.03	0.47±0.01	0.15±0.002	-	-

- = not detectable, the results are the mean ± SEM.

3.2 Tissue residues

Tissues concentrations of apramycin of slaughtered chickens following repeated oral (daily for five successive days) and intramuscular administration (daily for two successive days) of 25 mg/kg b.w.t. in broiler chickens were recorded in table 3 and table 4, respectively. The highest residues of apramycin were detected in kidneys followed by liver, lung, intestines, breast muscle and brain for both administration. No residues could be detected in lung, breast muscle, intestines and brain after 6 h and in liver and kidneys after 12 h, following intracrop dosing. After intramuscular injection, no residues could be detected in brain after 12 h and in other tissues (except kidneys) after 24 h. The data revealed a good distribution and penetration of apramycin in kidney, liver, and lung.

Apramycin, an aminocyclitol antibiotic, has been recommended for the treatment of intestinal bacterial infections in animals [1, 2, 19]. Pharmacokinetics of apramycin [5, 7, 8, 12] have already been established in poultry using microbiologic assay and the reported data are comparable with our results. One- or two-compartment models can be used to describe the apramycin serum concentration changes with time [4, 8, 20]. For mammalian species with a low body weight, the one-compartment model is more suitable [8]. Pharmacokinetic data of apramycin in birds are relatively few [8, 20]. The values for volume of distribution in the present study are in accordance with those observed in sheep, pigeons, rabbits and chickens [8]. A longer elimination half-life (1.67 h) was established in broiler chickens after i.v. injection [22] and a shorter half-life (0.25 h) was reported in pigeons [8].

The present study showed that the plasma concentrations of apramycin in chickens were greater than minimum inhibitory concentrations (MIC) of most sensitive organisms [1] after i.v. and i.m. administration of 25 mg/kg b.w.t.

Plasma concentration data were best fitted to a two-compartment pharmacokinetic model after i.v. dosing with a distribution phase completed by 0.33 h. Our

findings are similar to those reported in chickens [21] and calves [4, 20]. Apramycin was rapidly distributed in tissues after i.v. injection as indicated by the value of $t_{1/2\alpha}$ (1.55 h). On comparing this value with those of other aminoglycosides, it was relatively similar [23-26]. The biological elimination half-life ($t_{1/2\beta}$) of apramycin in chickens was 1.22, 2.31 and 2.10 h after lntracrop, i.m. and i.v. administration. The biological half-life of gentamicin was 30 min. in human [27] and 75 min. in dogs [16]; 61 min. in juvenile dogs [28]; 2.54 h in horses [29], and 1.61 h in calves [26]. This variation in elimination half-life of aminoglycosides in different species could be explained on the basis of variation of protein binding capacity of different drugs in different species [30].

The apparent volume of distribution at steady state of a drug (V_{dss}) is an indication of its diffusion in body tissue [30]. Apramycin showed high volume of distribution V_{dss} and V_{darea} of 4.85 and 5.55 litre/kg, respectively in chickens. These values were closely similar to those reported in chickens [21] and calves [4, 20, 22]. The relatively higher values of V_{dss} were indicative of a drug which was more extensively distributed in extravascular tissues. On the other hand, apramycin showed a high body clearance rate (1.9 litre/kg/h) in chickens which was consistent with its short elimination half-life value. Confirmation of this phenomenon was observed by higher Cl_{β} values for some aminoglycosides in dogs in relation to their lower $t_{1/2}$ values [31]. These values were higher than values of aminoglycosides such as kanamycin [31]; gentamicin [28, 29, 32] and apramycin in dogs [26], horse, man and calves for which (Cl_{β}) values were 0.24, 0.25, 0.35 and 0.88 litre/kg/h, respectively.

The bioavailability of apramycin after i.m. injection in chickens was higher with approximately 60% of it being absorbed. This value was similar to those observed in calves [26], which ranged from 60 to 66 % and in chickens, which was 85 % [21]. On the other hand, the bioavailability of apramycin after intracrop administration was very low (about 2.5 % of the drug being absorbed). This oral bioavailability of apramycin were lower than those found in other species and much lower than levels required for therapy. Therefore, no systemic therapeutic effect could be expected for this drug after oral administration in broilers. Similar results were reported and found that apramycin is normally not well absorbed from the intestinal tract of broiler chickens [6, 21].

The plasma protein binding capacity of apramycin in broiler chickens was low (only 25%). The highest concentrations of apramycin were present in the kidneys and liver after repeated oral (intracrop) and i.m. dosing. This observation was supported by Thomson *et al*., (1991) [6]. The high volume of distribution and low plasma protein binding capacity of apramycin in chickens is reflected by its stay in tissues for a longer time. This is due to the shorter half-life of drug elimination.

4. CONCLUSION

It is concluded that after i.v. and oral administration to broiler chickens, apramycin shows a pharmacokinetic behavior similar to that of aminoglycoside antibiotics.

5. REFERENCES

1. Ryden R and Moore BJ (1977). The in vitro activity of apramycin, a new aminocyclitol antibiotic. Journal of Antimicrobial Chemotherapy. 3: 609-613.
2. Walton JR (1978). Apramycin, a new aminocyclitol antibiotic. Journal of Antimicrobial Chemotherapy. 4: 309-313.
3. Theys H, Verhess I, Grimon A (1983). Apramycine tegen salmonellosis en colibacillosis bij mestkalveren. *Vlaams Diergeneeskd Tijdschr*. 52: 18-20.
4. Ziv G, Bor A, Soback S et al. (1985). Clinical pharmacology of apramycin in calves. *J. vet. Pharmacol. Therap.* 8: 95-104.
5. Freidlin PJ, Bock R, Bernath A et al. (1985). Apramycin: minimal inhibitory concentrations for avian Escherichia coli and serum levels after intramuscular injection in turkey. *J. vet. Pharmacol. Therap.* 8: 105-109.
6. Thomson TD, Cochrane RL, Donoho AL et al. (1991). Effects of intestinal pathology due to eoccidial infection on the oral absorption of apramycin in 4 week old broiler chickens. Proceedings of the 5th EA VPT Congress, Copenhagen, Denmark, pp. 275-277.
7. Shiha I (1987). Investigation of the influence of apramycin, its therapeutic levels, residues, withdrawal terms and cure of toxicosis in domestic animals. PhD thesis. Trakia University, Stara Zagora, Bulgaria.
8. Lashev L, Pashov DA. and Marinkov TN (1992). Interspecies differences in the pharmacokinetics of kanamycin and apramycin. *Veterinary Research Communications*.16: 293-300.
9. Lashev LD and Mihailov R (1994). Pharmacokinetics of apramycin in Japanese quails. 1. *Vet. Pharmacol. Therap.* 17:394-395.
10. Donos A (1982). Experimental use of apramycin against broiler colibacillosis. Bulletin of the Hellenic Veterinary Medical Society 33. 1: 65-70.
11. Afifi NA and Ramadan A (1997). Kinetic disposition, systemic bioavailability and tissue distribution of apramycin in broiler chickens. *Res Vet Sci.* 62: 249-252.
12. Lashev L (1998). Pharmacokinetics of apramycin and sulphachloryridazine coadministered with flavophospholipol and avoparcin to chickens. Bulgarian Journal of Veterinary Medicine. 1: 71–76.
13. Haritova AM, Djeneva HA, Lashev LD et al. (2004). Pharmacokinetics of gentamicin and apramycin in turkeys roosters and hens in the context of pharmacokinetic–pharmacodynamics relationships. *J. vet. Pharmacol. Therap.* 27: 381–384.
14. Cracknell VC, Andreotis J, Facibeni G et al. (1986). An evaluation of apramycin soluble powder for the treatment of naturally acquired Escherichia coli infections in broilers. *J Vet Pharmacol Ther.* 9:273-279.
15. Leitner G, Waiman R and Heller ED (2001). The effect of apramycin on colonization of pathogenic Escherichia coli in the intestinal tract of chicks. *Vet Quarterly*. 23: 62-66.
16. Baggot, J. D. (1978b). Some aspects of clinical pharmacokinetics in veterinary medicine 1. *J. Vet. Pharmacol. Therap.* 1: 5-18.
17. Bennett JV, Brodie JL, Benner EJ et al. (1966). Simplified, accurate method for antibiotic assay of clinical specimens. *Applied Microbiology* 14: 170-177.
18. Lorian V (1980). Antibiotics in Laboratory Medicine. Baltimore, Waverly Press. pp. 265-297.
19. Pankhurst JW, Diaz M, Zeri A et al. (1975). The treatment of disease in the young calf with apramycin. Proceedings of the 20th World Veterinary Congress Thessaloniki, Greece.
20. Shikha I (1987). Apramycin blood concentration and distribution in the body of calves after different methods of administration. *Vet Med Nauki.* 24: 64-71.
21. Aziza MA, Amer M, Saffaf AM et al. (1994). Pharmacokinetic profile and tissue distribution of apramycin in chickens. Veterinary Medical Journal of Giza. 42: 93-97.

22. El-gamal MHH (1992). Pharmacokinetics of ampicillin and apramycin in normal and pneumonic calves. M.Sc. (Pharmacology). *Thesis from Faculty of Veterinary Medicine, Cairo University.*
23. Chisholm GD, Calman JS, Waterarth PM et al. (1968). Distribution of gentamicin in body fluid. *British Medical Journal.* 2: 22-24.
24. Rodriguez V, Stewart D and Bodey GP (1971). Gentamicin sulfate distribution in body find. Clinical Pharmacology and Therapeutics. 11: 275-281.
25. Carbon C, Contrepoiss S and Lamotte-barrilson S (1978). Comparative distribution of gentamicin, tobramycin, sisomicin, netilmicin and amikacin in intestinal fluid in rabbits. *Antimicrobial Agents Chemotherapy.* 13: 368-371.
26. Aziz MA, El-bauomy AM, Abdel-motal SM et al. (1988). Bioavailability of apramycin in buffaloe calves. Zagazig Veterinary Journal. 16: 350-364.
27. Luff FC and Kleit SA (1974). Renal accumulation of aminoglycosides. *Journnal of Infectious Diseases.* 130: 656-660.
28. Riviere JE and Coppoc GL (1981). Pbarmacokinetics of gentamicin in the juvenile dog. American Journal of Veterinary Research. 42: 1621-1630.
29. Pedersoli WM, Belmonate AA, Purohit RC et al. (1980). Pharmacokinetics of gentamicin in horses. American Journal of Veterinary Research. 41: 351-355.
30. Gilman AG, Goodman LS, and Gilman A (1980). Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 6th edn. New York, Macmillan pp 21, Gorden, R. C.
31. Baggot JD (1978a). Pharmacokinetics of kanamycin in dogs. Journal of Veterinary Pharmacology and Therapeutics. 1: 163-170.
32. Gyselycneck AM, Forrey A and Cutler R (1971). Pharmacokinetics of gentamicin: distribution and plasma and renal clearance. *Journal of Infectious Diseases.* 124 S: 70-76.

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