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Effect of dose escalation and route of administration on tissue residues profile and withdrawal times of gentamicin and apramycin in broiler chickens

Hesham, S. Taha* and Hanaa M. Sultan**

Biochemistry, Toxicology and Feed Deficiency Dept., Pharmacology Unit* and Food Hygeine Dept.**, Animal Health Research Institute, Dokki, Egypt.

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Abstract

This study was conducted to determine the effect of dose escalation and route of administration on tissue residues profile of gentamicin and apramycin in broiler chickens. Gentamicin was administered (5 mg/ kg b.wt.) orally, intramuscularly (IM) and subcutaneously (SC) once daily for five days (Groups 1, 2 and 3). Apramycin was administered orally at doses 25 and 50 mg/kg b.wt. once daily for seven days (Groups 4 and 5).

The present study revealed that gentamicin still has antibacterial activity against *E. coli* and *klebsiella pneumoniae* where the MIC values were 0.195, 0.39 µg/ml respectively. Apramycin was effective in the treatment of *E.coli* and *Salmonella typhimurium* with MIC of 3.125 amd 6.25 µg /ml respectively. *Salmonella typhimurium* showed resistance against gentamicin. The serum protein-binding of gentamicin and apramycin were 4.60% and 21.74 % respectively.

A significant difference in the concentration of gentamicin in all organs between oral and IM or SC administration throughout the experiment was detected and the significant difference in the tissue concentration between IM and SC was observed in some tissues (heart, liver, kidneys, lung, intestine and brain) during the experiment. The presence of gentamicin residues in the liver after orally, intramuscular and subcutaneous administration were 0.65±0.02, 1.80±0.10 and 2.23±0.15 µg /gm and in the kidneys were 0.82±0.05, 2.30±0.30 and 2.80±0.10 µg /gm at the 1st day after the last dose respectively. No gentamicin residues above the MRL were detected in tissues at the 5th, 21th and 25th days after the last oral, I.M. and SC doses.

The results showed that apramycin residues not detected in all samples of muscle at the 1st day after the last oral doses of 25 and 50 mg/kg b.wt. Mean residues were significantly higher in kidneys (0.69±0.07µg /gm), brain (0.66±0.05 µg /gm), heart (2.10±0.09 07µg /gm) and intestine (0.68±0.07 µg /gm) of chicken administered 50 mg /kg b.wt. compared with those administered 25mg / kg b.wt. at the 1st day after the last oral dose. Apramycin residue was not detected at 3rd and 5th day after the last oral administration of a dose of 25 and 50 mg/kg b.wt. respectively except in the heart.

For gentamicin, withdrawal periods of 5, 21 and 25 days was recommended after oral, IM and SC administration. For apramycin, no need to establish withdrawal period for chickens according to the recommendations of EMA which stated that there is no need to establish MRLs for apramycin when administered at doses 25 or 50 mg/kg. b.wt for chickens.

It is recommended that when gentamicin is given to the broiler chickens by IM or SC injections, it should be during the first three weeks of age while for broiler breeder can be given gentamicin during breeding and production periods. For table-egg layers, gentamicin and apramycin can be given during the breeding period but not during the egg production period because MRL not established for eggs by the responsible authorites. Oral solution of gentamicin can be given to broiler, breeders and layers after following the recommended withdrawal period for edable tissues (5 days).

Moreover, using of a drug in a way or for a purpose not specified on the label whether by changing in the drug dose or in the route of administration, the withdrawal period of the drug must be firstly specified.

Key words *: Gentamicin, Apramycin, Residues profile, Withdrawal times.*

Introduction

In food-producing animals, besides the safety of the drugs, two main aspects must be guaranteed to ensure a proper pharmacological therapy. The first aspect concerns the demand to define dosing regimens suitable to achieve prefixed therapeutic objectives. The second aspect concerns the evaluation of the biotransformative pathways and the definition of the withdrawal time (WDT) necessary to assure the absence of residues higher than maximum residual limits (MRLs) in food-producing animals and so the healthiness of food destined to human consumption (**EMA, 1997)**.

To deliver safe food for human consumption, withdrawal times of pharmaceutical formulations of a drug must be fulfilled. In general terms, the WDT is the period of time required after completion of treatment needed for tissue concentrations of the drug and/or its metabolites to deplete to less than the established MRLs **(Riviere *et al.,* 1998)**.

The administration of one or another formulation of the same drug leads to violative concentrations of residues if individual withdrawal period are not considered. The final elimination phase depends on drug pharmaceutical formulation, dose, length of treatment, route and site of administration. In the extra vascular administration of a drug, the pharmaceutical formulation can condition the rate of absorption and consequently the final elimination phase. According to this, a formulation may require a longer WDT when the drug is slowly depleted from tissues. Otherwise, a shorter WDT can be used when faster depletion is adequately proven (**KuKanich *et al.,* 2005).**

Gentamicin is an aminoglycoside antibiotic indicated for the treatment of a variety of bacterial infections. It is normally used as the sulphate salt. In veterinary medicine gentamicin is used mainly as a solution for injection for chickens, turkeys dogs, cats and swine. In day-old chickens, gentamicin used for prevention of early mortality caused by *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* **(FDA, 2018**).

The committee for medicinal products for veterinary use recommended the extrapolation of maximum residue limits for gentamicin to all mammalian food producing species and fin fish**. (EMA, 2016).** Gentamicin is approved for use in horses (as injectable solution) and for poultry (as oral solution) **(EMA, 2015**). It is also used in human medicine, usually as a solution for injection for intramuscular administration. It is currently included in the list of essential medicines for human use of the World Health Organisation **(WHO, 2017).**

Gentamicin is widely used in the treatment of respiratory and enteric bacterial infections in animals including chickens **(Houdeshell *et al.,* 1982).** It is effective against aerobic Gram-negative microorganisms such as *Escherichia coli, Klebsiella, Pseudomonas, Salmonella* and some Gram-positive microorganisms such as *Staphylococcus aureus* **(Gilbert, 1991; Haritova *et al.,* 2004).** Aminoglycosides display concentration-dependent bactericidal activity (**Drusano, 2004).**

Gentamicin was found to be effective against many Gram-negative and positive susceptible bacterial species (**Conzelman, 1980)**. Gentamicin is indicated for *Pseudomonas aeruginosa* infections with few alternatives and approved for avian, bovine, camel, caprine, equine, rabbit, ovine and swine **(OIE, 2015).**

The pharmacokinetics of gentamicin has been studied in a variety of animal species such as cow **(Haddad *et al.,* 1986**), sheep **(Brown *et al.,* 1986**), turkey and rooster **(Pedersoli *et al.,* 1990**), hens **(Haritova *et al.,* 2004**) and chickens **(Abu-Basha *et al.,* 2007).**

Apramycin is a soluble aminocyclitol antibiotic. It is a mono-substituted deoxystreplamine compound produced by *Streptomyces tenebrarius*. Structurally, it is closely related to the aminoglycoside group of antibiotics

(O'Connor *et al.*, 1976).

It is used in the treatment of colibacillosis and salmonellosis in calves, bacterial enteritis in pigs, colibacillosis in lambs and *E. coli* septicaemia in poultry. It is also administered to rabbits. Apramycin is not authorised for use in laying birds nor for use in cattle or sheep producing milk for human consumption**.** The available products include an oral dose of apramycin intended for oral administration to poultry at a rate of 250 to 500 mg/liter (equivalent to 25 to 50 mg/kg b.wt.) for 7 days **(EMA, 1999).**

The pharmacokinetics of apramycin has been studied in a variety of animal species such as chicken **(Afifi and Ramadan, 1997)**, turkeys roosters and hens **(Haritova *et al.*, 2004)** and goats (**Toncho *et al.*, 2009)**.

There are no previous studies on the determination of withdrawal period of gentamicin when administering by oral administration or follow-up of gentamicin residues for long periods after intramuscular injection or SC administration (at the age of 20 days). Moreover, the effect of increasing the therapeutic dose of apramycin on the concentration of drug residues in the tissues is not previously studied. Accordingly, the main purpose of this study is to cover the above points and to evaluate the effect of escalating of the dose or changing the method of drug administration on the tissue residues profile and withdrawal period. As well as, the assessment of the efficacy of gentamicin and apramycin against the common pathogenic bacteria isolated from broiler chicken farms.

Materials and Methods

Experimental animals

Seventy two healthy broiler chickens, 20-25 days old, were used. Animals were accommodated in a suitable pen under hygienic condition with controlled temperature (25± 2°C), humidity (60±10%) and light (12 h) for at least a week before being used. Antibacterial-free food and water were available *ad libitum.*

Drug

1.**Gentamicin sulphate**: injectable solution in a glass vial, each 1 ml contain gentamicin sulphate 5gm (as sulphate). Manufacturer: Arab Company For Medical Products (Arabcomed) - Egypt, under trade name "**medgent 5%"**.
2.**Gentamicin sulphate**: oral solution in plastic bottle, each 1 ml contain gentamicin sulphate 160 mg. Manufacturer: Al-Mimas for veterinary drugs, Syria, under trade name " **Gentamas "**.
3.**Apramycin:** yellow powder in a plastic container. each 100 gm contains 86.5 gm of apramycin sulphate (equivalent to Apramycin base 59.5 gm) Manufacturer: WakiPharma- Egypt under trade name "**Apracure".**

Experimental design

The chickens were divided into five groups. The 1st, 2nd and 3rd groups were administered a single dose of gentamicin (5 mg/kg b.wt.) as oral, IM and SC administration, once daily for five consecutive days respectively. A fourth and fifth groups were administered apramycin (25 and 50 mg/kg b.wt.), once daily for seven consecutive days**.**

Analytical procedure

Blood samples (1–1.5 ml) were collected from wing vein of each chicken at:

- 24, 48, 72 and 96 h post the first dose (and 120 h, 144 h for apramycin).
- 1, 3 and 5 days after the last dose of the apramycin and gentamicin (oral.)
- 1, 3, 5, 7, 10, 18, 21 and 25 days after the last dose of gentamicin (IM and SC).

The samples were left to clot at room temperature then centrifuged at 3000 r.p.m for 15 min. to obtain clear serum and were kept frozen at -18°C until analyzed for drugs within a week.

Tissue residue study

At the end of the fifth and the seventh day of gentamicin and apramycin administration in broiler chicken respectively, three chickens were slaughtered at days: 1, 3, 5 days for apramycin and gentamicin oral and 7, 10, 18, 21 days for gentamicin IM and 25 days for gentamicin SC after the last dose of the drug (tables 5, 6, 7 and 8). Tissue samples from

heart, liver, kidneys, lung, stomach, intestine, thigh and breast muscles, spleen and brain were taken for drug assay.
One gram of organ was grinded with five milliter of distilled water and was then centrifuged at 1500 g for 15 min. **(San Martin *et al.,* 2007)**. Supernatants were transferred into dry tubes for drug assay.

Preparation of standard curves
By microbiological assay, standard curve of gentamicin and apramycin **(Fig. 1 and 2)** was done by using a stock solution of 250µg/ml of both drugs in distilled water and serum. Standard concentrations were obtained by further dilution in drug free healthy chicken serum or in healthy chicken homogenized tissues to obtain concentrations of 25, 12.5, 6.25, 3.25 and 1.56 µg /ml and using *E.coli* and *Bacillus subtilis* as test organisms respectively (**Afifi and Ramadan, 1997).**

Determination of antibiotic concentrations in serum and tissue
By the bioassay method using the standard curve of the drugs, gentamicin and apramycin concentrations in serum and tissue were measured (**Tsai and Kondo, 2001).**

Estimation of protein binding of the drug
According to (**Craig and Suh, 1980),** Gentamicin and apramycin were dissolved in distilled water and antibiotic-free chicken serum at different concentrations using *E.coli* for gentamicin and *Bacillus subtilis* for apramycin (Table 2 and 3). The differences in the diameter of the inhibition zone between the solution of the drug in the distilled water and serum samples were calculated. The percentage of protein-bound fraction was calculated according to the following equation:

$$\% \text{ Protein binding} = \frac{\text{Zone of inhibition in buffer} - \text{Zone of inhibition in plasma}}{\text{Zone of inhibition in buffer}}$$

Determination of minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC)
MIC and MBC of gentamicin and apramycin were determined against different organism, *Escherichia coli, Salmonella typhimuriun* and *Klebsiella pneumonia* which were obtained from serology unit in Animal Health Research Institute. Two fold dilutions of the antibiotic solution in nutrient broth were prepared using the culture of respective organism. The tubes were incubated at 37°C for 24 hours. The tubes were examined for growth and the lowest concentration showing no growth is MIC. For determination of MBC, all the tubes showing no bacterial growth in the MIC test were subcultured. A standard loopful from each tube was inoculated on nutrient agar plate. The plates are incubated at 35˚C for 18 hours **(Amita *et al.,* 2013).** The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculums. (table 1).

Statistical analysis
The results obtained were statistically analysed using Students t-test. Differences were considered significant when $P < 0.05$. All data are expressed as mean ± SD **(Guan *et al.,* 2014)**

Results
Detection of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)
The MIC/MBC of gentamicin against *E.coli, S. typhimurum* and *K. pneumoniae* were 0.195 / 0.195, 12.5 / 6.25 and 0.39 / 0.195 µg/ml while for apramycin were 3.125 / 1.56, 6.25 / 3.125 and 3.125 / 3.125 ug/ml respectively (table 1).

Estimation of the protein binding of the tested antibiotics:
In tables **(2 and 3)**, the obtained results showed that gentamicin and apramycin had low tendancy to bind with plasma protein of chicken's serum, the percentage of binding was 4.60 ± 1.53 and 21.74 ± 3.96 respectively.

Determination of serum concentration of gentamicin and apramycin:
Following oral, SC and IM administration of gentamicin (5 mg/kg b.wt.) and apramycin (25 and 50 mg/kg b.wt.) once daily for 5 and 7 consecutive days showed slight (non significant) increase in the serum concentration of the drugs after 48h, 72h and 96h post the first dose compared with 24h post the first dose. The concentrations of gentamicin in the serum after

oral, IM and SC administration were 0.92 ± 0.01, 1.36 ± 0.02 and 1.41 ± 0.02 µg /ml at 96h post the first dose respectively. Apramycin serum concentration was 0.97 ± 0.04 and 0.85 ± 0.17 µg /ml at 96 h post the first dose of oral administration of 50 and 25 mg/kg b.wt. respectively.

Tissue residues of apramycin in broiler chickens:

Tissue concentrations of apramycin in slaughtered chickens following oral administration of 25 and 50 mg / kg b.wt. once daily for 7 consecutive days are recorded in (table 5). The present data revealed that apramycin concentration in liver and kidney were 0.22±0.0.2 and 0.39±0.02 µg /gm at the 1st day after the last dose of 25 mg/kg.b.wt. while were 0.28±0.01, and 0.69±0.07µg /gm after administration of 50 mg/kg b.wt. respectively. Apramycin (25 and 50 mg / kg b.wt.) disappeared after 3 and 5 days after the last dose except in the heart.

Tissue residues of gentamicin in broiler chickens:

In tables (6, 7 and 8), gentamicin was distributed in all tissues after oral, IM and SC administration. The tissue level concentrations were highest in the heart, kidneys, brain and liver. In the liver and kidney, gentamicin residues were detected at the 1st day after the last oral dose at a concentration of 0.65±0.02 and 0.82±0.05 µg/gm while the concentration after IM administration were 1.80±0.1 and 2.30±0.30 µg/g. After SC administration, the drug was detected at 25 days post administration in intestine (0.10±0.01 µg/ml). There are a significant differences in the level of residues in all organs between oral and IM or SC administration. On the other hand, a significant differences between the concentration of the residues in some organs between IM and SC administration during the experiment.

Discussion

Gentamicin is a broad-spectrum antibiotic of the aminoglycoside group. Gentamicin is currently authorised in more than 25 countries worldwide and it is a medically necessary product (**WHO, 2017**).

Gentamicin is a polar hydrophilic drug that distributed into the extracellular space with poor tissue penetration and accumulates in the tissues of high lipid content (**Ziv *et al.*, 1982; Frazier *et al.*, 1988**). It is excreted unchanged from the body, primarily by renal glomerular filtration (**Al-Amoud *et al.*, 2002).** Gentamicin is a very polar entity that does not undergo metabolism in the body and is excreted mainly by glomerular filtration (**Zaske, 1992**).

In vitro susceptibility testing by determination of MIC to study the effectiveness of antibiotic against specific pathogen. Moreover, the recent increase in incidence of multidrug resistant bacterial infections has increased treatment complexity and some bacteria are resistant to many different antibiotics; they are multidrug-resistant. Multidrug-resistant bacteria can be difficult to treat and facilitates spread of antibiotic resistance. Susceptibility testing must be routinely performed for selecting an effective antibiotic.

In the present study, the in-vitro antibacterial activity of gentamicin against bacteria of high incidence in poultry farms were evaluated and the determined MIC values were 0.195, 12.5, and 0.39 µg/ml while the MBC were 0.195, 6.25 and 0.195 µg/ml against *E.coli, Salmonella typhimurium* and *Klebsiella pneumonia* respectively. Using the available *Enterobacteriaceae* breakpoints, *Salmonella typhimurium* showed resistance to gentamicin while *E.coli* and *K. pneumoniae* has been shown to be susceptible to gentamicin where, MIC breakpoints were ≤ 4 µg /mL for susceptible organisms and ≥ 4 µg /mL for resistant organisms (**EUCAST, 2019)**.

These results are in consistent with values reported by (**Abu-Basha *et al.*, 2012**), who found that MIC of gentamicin was 1µg /ml against *E.coli* while MIC was found to be < 0.5 µg /ml against *k. pneumoniae* **(Tang *et al.*, 2016)**. In a study conducted by **Mandal *et al.* (2009)** who found that MICs of gentamicin was 0.01– 4 µg/mL against *Salmonella* enteric serovar Typhi isolates from 1991 to 2003 which means that bacteria acquire resistance against gentamicin.

To evaluate the efficacy of apramycin against the above bacteria, the MIC of apramycin were 3.125, 6.25, 3.125 µg /ml and the MBC were 1.56, 3.125 and 3.125 µg/ml against *E.coli, Salmonella typhimurium* and *K.pneumoniae* respectively. Depending on the CLSI (Clinical and Laboratory Standards Institute) breakpoints, *E.coli* and *Salmonella typhimurium* has been shown to be susceptible to apramycin where MIC breakpoints were ≥ 32 µg /mL for resistant organisms **(CLSI, 2013)**.

It is well known that no breakpoint for apramycin against *k. pneumoniae* has been defined by European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical and Laboratory Standards Institute (CLSI), the efficacy of apramycin against *K. pneumoniae* is difficult to assess. Further studies are required to establish the breakpoint of apramycin against *K. pneumoniae.*

In pevious studies, MIC values of apramycin against *E.coli* was 1 µg/ml **(Kobe *et al.,* 1996).** MIC_{50} and MIC_{90} of apramycin against Carbapenem-resistant *Enterobacteriaceae* (CRE) was found to be 4 and 8 µg/ml, respectively **(Smith and Kirby, 2016)**. Apramycin MIC against *K.pneumonia*e was reported as 4 µg /ml (**Juhas *et al.,* 2019**).

Bacterial resistance to antibiotics is rising and so it must be of interest to the researchers and the competent authorities to prevent the transmission of these resistance strains to human via the food chain. In addition, using antibiotics routinely to promote growth and prevent disease in healthy animals must be stopped **(WHO, 2017)**. Moreover antibiotics must be used under veterinary supervision and a reliable antimicrobial susceptibility testing can be performed before the beginning of treatment to avoid the development of resistance to antibiotic.

Protein-binding of antibiotics (PB) affects the drug clearance from the body. PB may affect the efficacy of antimicrobial therapy as the non -protein-bound fraction of a drug in plasma can penetrate into and equilibrate with the extravascular space. Penetration into the extravascular space is highly important for antimicrobial therapy, as the majority of bacterial and fungal infections occur in the interstitial fluid of tissues or in other body fluids than blood **(Fasano *et al.,* 2005)**.
In-vitro serum protein binding percent of gentamicin in chicken's serum was 4.60%. This finding is in accord with that reported in broiler chickens by **Abo-EL-Sooud *et al.,* (2012) (**3.4%**)**. In this respect, **Zaske (1992)** stated that the plasma protein binding of gentamicin (aminoglycosides in general) is less than 10%. In other studies , the plasma protein binding of gentamicin have ranged from zero binding (**Rosenkranz *et al.,* 1978)** to 20% binding **(Meyers *et al.,* 1978).**

In this study, The serum protein-binding of apramycin was 21.74 %. The value may be close to that reported in chicken's serum , 26% (**Afifi and Ramadan, 1997)** and in chicken's serum, 25% **(Elbadawy and Aboubakr, 2017).**

Serum concentration data of gentamicin and apramycin after repeated administration for five and seven days respectively revealed slight increase in a drug serum levels (non significantly) which proves a slight accumulation of drugs in serum. Following the repeated oral, IM and SC administration of 5 mg /kg b.wt. of gentamicin, serum levels at 96 h post the first dose of the drug were 0.92±0.01, 1.36±0.02 and 1.41±0.02 µg/ml respectively compared to the serum level at 24h post the first dose of the drug (0.85±0.02, 1.22±0.03 and 1.27±0.06 µg / ml respectively). Apramycin serum concentration after repeated oral administration of 25 and 50 mg/kg b.wt. for seven days were 0.85±0.17 and 0.97±0.04 µg/ml at 96 h post the first dose compared to the serum level at 24 h post the first dose of the drug (0.74±0.17 and 0.86±0.07ug/ml).

These results are in consistent with values reported in dogs **(Howard *et al.,* 1977)** who found that there was no evidence of accumulation or altered plasma concentrations of apramycin after prolonged treatment with oral doses of 25, 50 and 100 mg/kg b.wt. In addition, gentamicin administered at 1 mg/kg every

eight hours for the usual 7 to 10 day treatment period to patients with normal renal function does not accumulate in the serum **(FDA, 2013).**

In the present study ,chickens were administered with gentamicin at the recommended dose (5 mg/kg of bodyweight) for 5 days by different routes showed a significant difference in the concentration of the drug in all organs throughout the experiment when comparing oral administration with IM or SC injections while the significant difference between IM and SC observed in some organs (heart, liver, kidneys, lung, intestine and brain) during the experiment.

The residue testing revealed high concentrations of oral gentamicin in heart (0.93 ± 0.08 µg /gm) followed by kidney (0.82±0.05 µg / gm), brain (0.80±0.15 µg /gm) and liver (0.65±0.02 µg /gm) at the 1st day after the last dose. Moreover, the drug could not be detected in organs after 5 days of the last dose except in the brain (0.10±0.02 µg/g) and the heart (0.10±0.01 µg/g).

Results of IM administration showed that kidney and liver contained drug concentrations (2.3±0.03 µg /gm, 1.8±0.10µg/g respectively) at the 1st day after the last dose. Gentamicin residues were detected in heart (0.27±0.03µg / gm), kidney (0.28±0.04µg /gm), intestine (0.35±0.11µg /gm) and brain (0.42±0.04 µg / gm) at the 18th day after the last administration. The drug could not be detected by microbiological assay in all tested tissues except in intestine (0.10±0.04 µg /gm) and brain (0.10±0.01 µg /gm) at the 21th day after the last dose of drug.

After SC administration, tissue concentrations of gentamicin of slaughtered chickens following repeated administration were evaluated. The highest residues of the drug were detected in heart (3.03±0.30 µg /gm) followed by kidneys (2.80±0.10 µg /gm), brain (2.13±0.15µg / gm), liver (2.23±0.15) at the 1st day after the last dose. No residues were detected in the breast, stomach and spleen at the 18th day after the last dose. Gentamicin residue was detected in heart, kidney, intestine and brain at the 21st day after the last dose of drug while the residues still detected in intestine only (0.10±0.01) at the 25th day after the last dose.

The result was closely similar to that previously reported by (**Alm El Dein and Elhearon, 2010**) who concluded that the laying hens injected with gentamicin, the eggs which produced during the withdrawal period which reach up to 12 and 15 days for doses 2 mg/kg b.wt. (IM) and 4 mg/kg b.wt. (SC) respectively must be discarded because it was evident that eggs were contained gentamicin residues which have harmful effect on the consumers.

In a study conducted by (**Abo-EL-Sooud *et al.*, 2012)**, gentamicin residues were not detected in tissues and serum of broiler chickens (40-45 days old) after 12 h except in the liver and kidneys after injection with a single dose of 5 mg /kg b.wt. by IM routes (in the thigh and pectoral muscles). For the liver and kidneys, gentamicin residues were found after 48 h.

In a similar study carried out by **(Filazi *et al.*, 2005)**, the residues of gentamicin in the whole egg were detected till 9 day after the last I/M or SC dose of 10 mg/kg b.wt. administered to the laying hens. The Canadian gFARAD (global food animal resistance avoidance databank) will continue to discourage the extralabel use of gentamicin in food producing animals and because of the accumulation phenomenon in the kidney, multiple doses, rather than single high doses, will incur the highest residues and the longest withdrawal interval recommendations **(Doling, 2006).** In this respect FDA reported that do not slaughter chickens for food for at least 5 weeks after the last SC injection of day-old chickens by 0.2 mg of gentamicin / chicken, **(FDA, 2018)**.

The above results indicate that the repeated administration of gentamicin by different routes revealed that it has a good distribution in all tissues including the brain. The high volume of distribution (**Abo-EL-Sooud *et al.*, 2012)** and low protein binding of this drug in chickens is reflected by its persistence in tissues for longer periods by IM and SC routes. **Brown *et al.* (1985)** explained the accu-

mulation of gentamicin is may be due to a slow release from tissues containing high concentrations. Such high concentration may be achieved by active uptake by the proximal tubules and other body tissues **(Schentag and Jusko, 1977).**

A tolerance of 0.1 part per million (0.1 µg / gm) is established for negligible residues of gentamicin sulfate in the uncooked edible tissues of chickens and turkeys **(FDA, 2018)**. A microbiological ADI of 4 µg/kg b.wt. (240 µg/ person) was established as the overall ADI for gentamicin (**EMA, 2016**).
The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an ADI of 0-20 µg/kg b.wt. on the basis of a microbiological end-point. The Committee noted that the lowest NOEL (no-observable-effect level) identified at the forty-third meeting in toxicological studies was 10 mg per kg bw, which is 500 times the microbiological ADI. **(WHO, 1998**).
Based on the tolerance level, a withdrawal periods of 5, 21, 25 days was recommended after oral, IM. and SC administration.

The difference in the chicken age at the time of drug administration may explain the variation in withdrawal period between our results (25 days) and FDA recommendation (5 weeks) where the pharmacokinetic of drugs was influenced by the age mainly with regards to the persistence of blood level, taking into consideration the lack of metabolism, the observed tendencies could be connected with the function of the kidney as a primary route for drug excretion, including their metabolism (**Haritova *et al.*, 2003)**

The presence of apramycin residues in serum and tissues after administration of a single oral dose of 25 and 50 mg /kg b.wt. daily to broiler chickens for seven days was studied. The concentrations of apramycin residues was signicantly higher in chickens administered 50 mg /kg b.wt. than those administered 25 mg/ kg b.wt. in the heart (1.56±0.13 and 2.10±0.09 µg /gm), kidney (0.39±0.02, and 0.69±0.07 µg /gm), intestine (0.34±0.03 and 0.68±0.07 µg /gm) and the brain (0.41±0.03 and 0.66±0.05 µg /gm) at the 1st day after the last dose respectively while there is a significant increase in the residue level in the heart between the two doses at the 3rd day after the last dose. No apramycin residues were detected in tissues at the 3rd and 5th days after the last dose of 25 and 50 mg/kg except in the heart (0.26±0.03, 0.42±0.02 µg /gm respectively).

The reported residues of apramycin in our studies was similar to that previously reported in chickens **(EL. Sayed *et al.*, 2018)** after oral administration of 25 mg/kg b.wt. three times daily for 5 consecutive days, apramycin residues were detected in kidneys (1.00±0.005 µg/ ml), liver (0.190±0.005 µg/gm), intestine (0.183±0.008 µg /gm) and breast muscle (0.106±0.012µg /gm) and the treated chickens must not be slaughtered before 3 days from last dose of repeated administration of apramycin to withdraw the drug residues from all tissues of treated chickens. **Afifi and Ramadan (1997)** concluded that poultry farms must give at least two days premarketing withdrawal time for apramycin to ensure that the drug is eliminated from the tissues. The above recommendation of withdrawal period not based on scientific facts such as the establishment of MRL.

Our result are different from those of **(Elbadawy and Aboubakr, 2017)** who found that no apramycin residues were detected in tissues after 6 h except in liver and kidneys following repeated intracrop dosing of 25mg/kg b.wt daily for five days while the residue was detected in the kidneys at 6 days after the last IM dose of 25 mg/kg b.wt for 2 succesive days in chickens. Furthermore**, Filazi *et al.* (2005)** pointed out that there was a direct correlation between drug dosage and the concentration of drug residue.

European Medicine Agency (EMA) approved apramycin for bovine, sheep, chickens, rabbits and pigs with the acceptable daily intake (ADI) of 40 µg /kg b .wt. (i.e. 2400 µg /person) and the MRLs was 1000 µg/kg in muscle and fat of bovine.
EMA reported that broilers whose given drinking water containing 559 mg apramycin sulphate / liter for 5 days, the residues in all sam-

ples of muscle were less than 50 µg /kg and residues in liver were variable between individual birds and were 260 to 540 µg /kg on the day of treatment. Within one day of the end of oral treatment of apramycin, the intake of total residues from chicken tissues represents approximately 6% of the ADI and so the committee for veterinary medicinal products concludes that there is no need to establish MRLs for apramycin for chickens and recommends its inclusion in annex II of council regulation (EEC) No 2377/90 (**EMA, 1999)**. In addition, the codex committee on residues of veterinary drugs in foods (CCRVDF) recommended temporary MRLs at 5 mg/kg only in cattle and chicken kidney, measured as apramycin based on statistical approaches **(FAO, 2012).** FDA approved apramycin for swine only with tolerance level of 0.1 part per million **(FDA, 2017)**. Morever, apramycin is not used in human medicine.

Based on the above recommendation, although there is a significant difference in the concentration of residues of apramycin in the tissues when the dose is changed from 25 to 50 mg/kg b.wt., a zero withdrawal period was recommended for the two doses but it is not advised to give apramycin to laying hens where MRL is not established for eggs.

The tissue residue profile and the withdrawal period of the drug must be specified before escalation of dose or changing in the route of administration or the use of an approved drug in a manner that is not in accordance with the approved labeling (the extra-label use) when the health of an animal is threatened, or when suffering or death may result from failure to treat animals

Conclusion

Gentamicin and apramycin are distributed almost in all tissues of broiler chickens. After oral, IM and SC administration of gentamicin at a dose of 5mg/kg b.wt once daily for 5 days, a withdrawal periods of 5, 21 and 25 days were recommended respectively. Due to the longest withdrawal period of gentamicin when given by IM or SC routes, gentamicin can be given to broiler chickens during the first three weeks of age.

No withdrawal period was required for apramycin when administered at doses 25 or 50 mg/kg. b.wt. for chickens. Gentamicin and apramycin not given to laying hens producing eggs for human consumption. Gentamicin can be used in treatment of infections caused by *Escherichia coli.* and *K. pneumoniae* while Apramycin for *Escherichia coli.* and *Salmonella typhimurium* infections. Any changes in the dosage regimen, the withdrawal period must be determined firstly.

Table (1). Minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) of gentamicin and apramycin (µg/ml) against different organisms.

Antibiotic	Organism, MIC/MBC (µg/ml)		
	E.coli	***S. typhimurium***	***K.pneumonea***
Gentamicin	0.195/0.195	12.5/6.25	0.39/0.195
apramycin	3.125/1.56	6.25/3.125	3.125/3.125

Table (2). In -vitro protein binding percentage of gentamicin in chicken´s serum.

Concentration (µg/ml)	Average corrected values of Concentrations inhibition zones (mm)		
	Serum	**Distilled water**	**Protein binding %**
25	25.50±0.5	26.67 ±0.29	4.39
12.5	23.33±0.58	24.65±0.58	5.35
6.25	21.66±0.76	22.17±0.57	2.30
3.125	19.33±0.28	20.67±0.29	6.48
1.56	17.83±0.58	18.67±0.29	4.49
Mean ± S.D.	4.60±1.53		

Table (3) . In -vitro protein binding percentage of apramycin in chicken´s serum.

Concentration (µg/ml)	Average corrected values of Concentrations inhibition zones (mm)		
	Serum	Distilled water	Protein binding %
25	**18.67±**	**23.0 ±1.5**	**18.82**
12.5	16.0±0.28	21.33 ± 0.58	24.98
6.25	13.83±0.76	18.17±0.29	23.88
3.125	11.66±0.57	15.5± 0.5	24.77
1.56	10.83±0.29	12.93±0.11	16.24
Mean ± S.D.	21.74± 3.96		

Table (4). Serum concentration of gentamicin and apramycin (µg/ml) after repeated dosing for five and seven days in chickens respectively (n=3).

Antibiotic	Post the first dose by					
	24 h	48h	72h	96h	120 h	144 h
Gentamicin (oral) - 5mg/kg b.wt.	0.85±0.02	0.87±0.03	0.89±0.03	0.92±0.01	---	---
Gentamicin (IM) - 5mg/kg b.wt.	1.22±0.03	1.28±0.04	1.31±0.07	1.36±0.02	---	----
Gentamicin (SC) - 5mg/kg b.wt.	1.27±0.06	1.33±0.05	1.39±0.05	1.41±0.02	---	---
Apramycin (oral - 50mg/kg b.wt.)	0.86±0.07	0.92±0.05	0.95±0.02	0.97±0.04	0.98±0.02	0.99±0.05
Apramycin (oral – 25 mg/kg b.wt.)	0.74±0.17	0.78±0.01	0.81±0.03	0.85±0.17	0.86±0.17	0.88±0.17

(*) : No significance was detected between the results of 48,72,96,120,144 h in comparison with 24 h post the first dose. (---) : not required where gentamicin was administerd for 5 days only .

Table (5). Serum and tissue concentrations of apramycin (ug/ml or µg/gm) following repeated oral administration of 25 or 50 mg/kg b.wt. once daily for 7 consecutive days (n=3).

Dose	25mg/kg b.wt.			50 mg/kg b.wt		
Tissue	Time of slaughter after the last dose (days)			Time of slaughter after the last dose (days)		
	1st	3rd	5th	1st	3rd	5th
serum	0.51± 0.10	ND	ND	0.71± 0.20	ND	ND
Heart	1.56 ± 0.13	0.26 ± 0.03	ND	2.10 [a]± 0.09	1.2 [a] ± 0.10	0.42 ± 0.02
Liver	0.22 ± 0.02	ND	ND	0.28± 0.01	ND	ND
kidney	0.39±0.02	ND	ND	0.69[a] ± 0.07	0.35±0.02	ND
lung	0.29±0.03	ND	ND	0.31± 0.02	ND	ND
stomach	ND	ND	ND	ND	ND	ND
intestine	0.34±0.03	ND	ND	0.68 [a] ±0.07	0.34±0.19	ND
thigh M.	ND	ND	ND	ND	ND	ND
Breast M.	ND	ND	ND	ND	ND	ND
Brain	0.41±0.03	ND	ND	0.66[a] ±0.05	0.36±0.04	ND
spleen	ND	ND	ND	ND	ND	ND

([a]) Represents the significance between doses of 25 and 50 mg/kg b.wt at the same organs and day ND : Not detected

Table (6). Serum and tissue concentrations of gentamicin (ug/ml or µg/gm) following repeated oral administration of 5 mg/kg b.wt. once daily for 5 consecutive days (n=3). (Mean ± SD)

Tissue	Time of slaughter after the last dose (days)		
	1st	3rd	5th
Serum	0.91±0.03[bc]	0.33±0.01[bc]	ND
Heart	0.93±0.08[bc]	0.46±0.03[bc]	0.10±0.01[bc]
Liver	0.65±0.02[bc]	0.28±0.04[bc]	ND
kidney	0.82±0.05[bc]	0.31±0.03[bc]	ND
Lung	0.38±0.03[bc]	0.16±0.01[bc]	ND
stomach	0.21±0.02[bc]	ND	ND
Intestine	0.40±0.03[bc]	ND	ND
Thigh muscle	0.39±0.07[bc]	ND	ND
Breast muscle	0.31±0.06[bc]	ND	ND
Brain	0.80±0.15[bc]	0.58±0.03[bc]	0.10±0.02[bc]
Spleen	0.22±0.03[bc]	0.15±0.01[bc]	ND

([b, c]) : Represents the significance in comparison with IM And SC administration at the same organs and day respectively .
ND : Not detected

Table (7). Serum and tissue concentrations of gentamicin (ug/ml or µg/gm) following repeated I.M. administration of 5 mg/kg b.wt. once daily for 5 consecutive days (n=3). (Mean ± SD).

Tissue	Time of slaughter after the last dose (days)						
	1st	3rd	5th	7th	10th	18th	21th
Serum	1.26±0.02	0.52±0.04	ND	ND	ND	ND	ND
Heart	2.46±0.05[d]	1.85±0.13 [d]	1.40±0.08	0.90±0.05 [d]	0.61±0.03 [d]	0.27±0.03 [d]	ND
Liver	1.80±0.10	1.28±0.01[d]	0.95±0.07	0.65±0.03	0.39±0.02 [d]	ND	ND
Kidney	2.30±0.30[d]	1.65±0.05[d]	1.15±0.01 [d]	0.85±0.02	0.52±0.03[d]	0.28±0.04 [d]	ND
Lung	1.70±0.10 [d]	1.13±0.35 [d]	0.90±0.03	0.72±0.04	0.40±0.02	ND	ND
Stomach	0.77±0.09	0.50±0.04	0.32±0.03	0.21±0.02	0.12±0.02	ND	ND
Intestine	1.65±0.2 [d]	1.25±0.08 [d]	0.98±0.01	0.75±0.04	0.55±0.03	0.35±0.11	0.10±0.04
Thigh muscle	1.78±0.1	0.88±0.05	0.59±0.02	0.39±0.02	0.19±0.02	ND	ND
Breast muscle	0.84±0.09	0.62±0.04	0.42±0.03	0.29±0.06	ND	ND	ND
Brain	2.10±0.3	1.68±0.03	1.25±0.05 [d]	0.83±0.07 [d]	0.64±0.03	0.42±0.04	0.10±0.01
Spleen	0.63±0.05	0.49±0.04	0.31±0.01	0.20±0.04	0.11±0.02	ND	ND

([d]) : Represents the significance in comparison with SC administration at the same organs and day respectively .
ND : Not detected

Table (8). Serum and tissue concentrations of gentamicin (ug/ml or µg/gm) following repeated S.C. administration of 5 mg/kg b.wt. once times daily for 5 consecutive days (n=3). (Mean ± SD).

Tissue	Time of slaughter after the last dose (days)							
	1st	3rd	5th	7th	10th	18th	21th	25th
Serum	1.29±0.03	0.64±0.01	0.14±0.01	ND	ND	ND	ND	ND
Heart	3.03±0.30	2.5 ± 0..25	1.86±0.10	1.56±0.10	1.36±0.11	0.90±0.05	0.30±0.04	ND
Liver	2.23±0.15	1.65±0.08	1.15±0.02	0.88±0.01	0.62±0.0.3	0.30±0.02	ND	ND
Kidney	2.8 ± 0.10	2.20±0.05	1.68±0.20	1.27±0.10	1.18±0.27	0.57±0.07	0.31±0.02	ND
Lung	2.33 ± 0.35	1.70±0.20	1.10±0.15	0.95±0.30	0.75±0.04	0.35±0.01	ND	ND
Stomach	0.84±0.01	0.62±0.03	0.39±0.04	0.27±0.03	0.15±0.01	ND	ND	ND
Intestine	2.35±0.02	1.83±0.10	1.44±0.05	1.23±0.04	1.03±0.03	0.88±0.11	0.48±0.01	0.10±0.01
Thigh muscle	1.90±0.05	1.2±0.02	0.65±0.13	0.45±0.03	0.22±0.02	0.10±0.03	ND	ND
Breast muscle	1.11±0.12	0.75±0.04	0.52±0.02	0.32±0.01	0.20±0.01	ND	ND	ND
Brain	2.13±0.15	1.90±0.05	1.60± 0.30	1.42±0.10	0.95±0.21	0.46±0.02	0.28±0.03	ND
Spleen	0.67±0.02	0.51±0.03	0.33±0.02	0.28±0.02	0.15±0.03	ND	ND	ND

ND : Not detected

Figure (1). Standard curve of gentamicin in distilled water ( ) and normal chicken serum ( ).

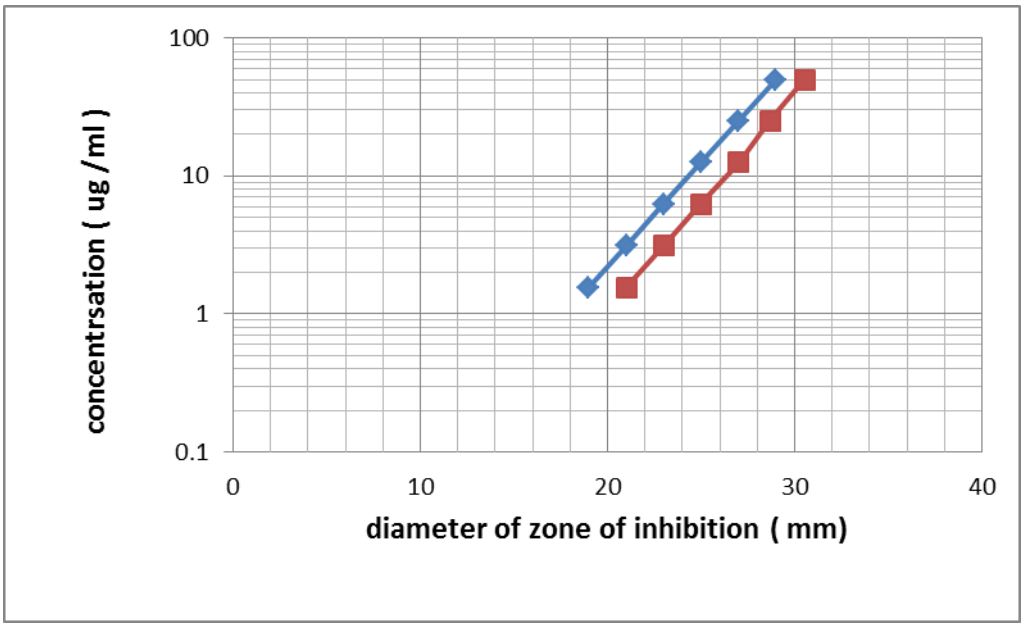
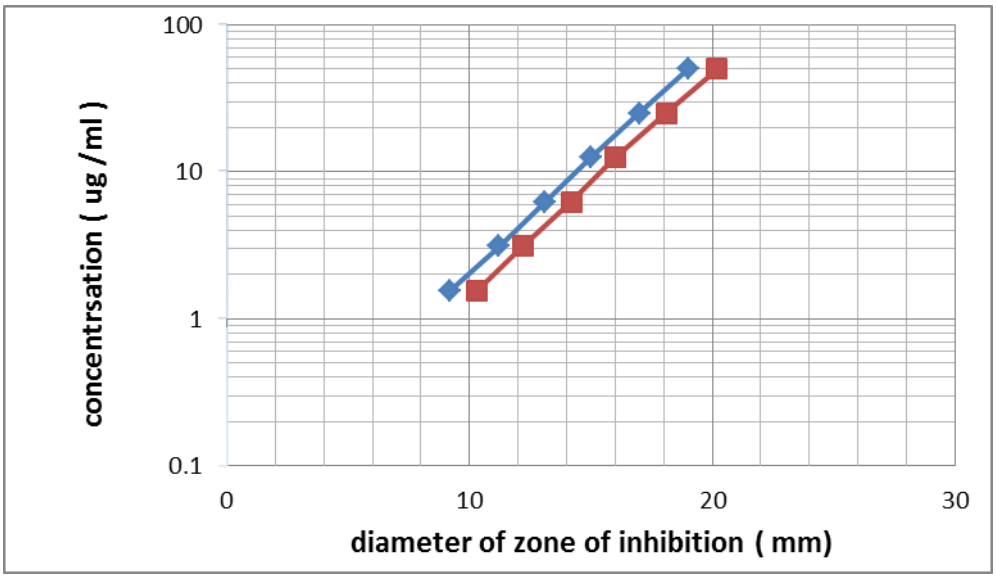


Figure (2). Standard curve of apramycin in distilled water ( ) and normal chicken serum ( ).



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