Effect of Potassium Citrate on Cephradine Residues in Broiler Chicks

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Abstract:

The present study was established to estimate Cephradine residues in the serum and tissue of broiler chicks following oral administration of Cephradine (Atocef Forte®) and also to detect the effect of potassium citrate on Cephradine residues in broiler chicks. A total number of 40 Hubbard chicks of 21 days old were used in this study after dividing into 2 equal groups (each of 20 chicks). Cephradine (Atocef forte®) was given an oral dose of 15mg\kg body weight twice daily for five consecutive days to the group (1) and was given twice daily for 3 days but at last 2 days, Cephradine was given for 12hr, potassium citrate for another 12h in the group (2). Cephradine was estimated in serum, liver, kidney, and breast muscle of broiler chicks at 24, 48, 72, 96hr post the last dose by using HPTLC assay. Residues study revealed a good absorption and distribution of Cephradine in the liver, kidney and, breast muscle. Cephradine concentrations could be detected in serum till 2d day, in breast muscle till 3d day but in liver and kidney till 4th day. On the other hand, the group that takes both Cephradine and potassium citrate caused a significant decrease in drug concentration in serum, liver, kidney, and breast muscle. It could be concluded that Cephradine can be detected in the kidney, liver at the highest concentration with the lowest concentration in serum and breast muscle. Potassium citrate is effective in reducing Cephradine residues in broiler chicks.

KEYWORDS: Potassium citrate, Cephradine, Residues, Broiler

Introduction

Poultry production is one of the most common food industries in the world. ***(FAO, 2017a)*** Most countries use a wide range of antimicrobials to raise poultry.

(Landers et al., 2012; Boamah et al., 2016). A large number of such antimicrobials are important in human medicine ***(WHO, 2010; WHS, 2017).*** Antibiotics have aided in the efficient production of poultry by improving growth, feed efficiency, and disease prevention, allowing consumers to buy high-quality meat at a cheaper price. Despite these advantages, consumers believe that edible poultry tissues could be contaminated with dangerous levels of drug residues. ***(Donoghue, 2003).***

The administered veterinary drug tends to accumulate in high concentrations in the edible tissues of treated chicken ***(Seri, 2013).*** The majority of these residues consist of parent and derivative compounds (or both), as well as metabolites, conjugates, and remnants bound with macromolecules ***(Alhendi et al., 2000; Alm-El-Dein & Elhearon, 2010).*** Ingestion of tissues and organs (meat, offal, eggs, etc.) containing drug residues above safe maximum residual levels (MRLs) can potentially pose serious public health hazards ***(Muhammad et al., 2007; Nisha, 2008; Seri, 2013).***

Cephalosporin antibiotics are a well-known and effective class of antibiotics used in veterinary medicine to prevent and treat bacterial infections ***(Becker et al., 2004).*** They're known β -lactam antibiotics because they all have the same structural feature: the β -lactam ring. Clinically, they are classified into four-generation based on their antimicrobial activity range ***(Sweetman, 2009).*** Cephradine is a first-generation cephalosporin with a wide antibacterial spectrum against gram-positive and gram-negative bacteria. it has worldwide approval for a variety of human diseases, as well as approval in many countries for the treatment of a variety of diseases in broiler chicks. ***(Tollefson & Karp, 2004).*** Based on the conclusions of several studies that the oral bioavailability of Cephradine is excellent and is recommended to be used in controlling *Salmonella Enteritidis or* colisepticemic infections in broilers ***(EL-Sayed et al., 2016; Aboubakr and Elbadawy, 2017; Abdel-Alim and Kawkab 2020),*** This study was designed to study tissue concentration of Cephradine in chick's tissues and to propose withdrawal time necessary to ensure that the treated animals are free from potentially harmful residues that may reach the human food chain. Additionally, we attempt to shorten the withdrawal time using potassium citrate.

MATERIALS AND METHODS

1. Experimental Drugs

1.1. Cephradine

Cephradine, a water-soluble powder. Each 100 gm of powder contains a 20gm Cephradine base. It is available as a package of 250 gm of powder. It is produced by ATCO pharma for pharmaceutical industries company, Egypt. Under the trade name (Atocef Forte®). Dose: 0.5 gm / Liter in drinking water.

1.2. Potassium citrate:

Potassium citrate is dispensed as water-soluble powder, it is available as a package containing 1kg of powder, it is produced by UCCMA (United Company for Chemical and Medical Preparation). Dose: 1gm / Liter in drinking water.

2. Experimental birds

A total number of forty healthy Hubbard chicks with an average body weight of 600:700gm (21 days old). Chicks were of both sexes. they were chosen randomly from poultry farm from Abo Sewer Ismailia Government, Egypt. Chicks were fed a commercial balanced ration and were kept ten days prior to the experiment to ensure that all drugs were completely excreted from the bodies of the chicks. chicks were given a balanced formulated diet free from any additives.

3. Experimental design

The experimental birds were grouped into 2 identical groups:

Group (1): was given Cephradine orally 15 mg/Kg B.Wt. daily for five consecutive days.

Group (2) was given Cephradine orally 15mg/kg B. Wt. daily for 3 days, but in the last 2 days give Cephradine in the morning and potassium citrate in the afternoon.

4. Collection of samples

4.1. Blood samples

About one milliliter of blood was collected from the left-wing vein in 24, 48, 72,96 hours following the last dose in a sterilized tube (without any anticoagulant) and let to clot at room temperature. The blood sample was centrifuged for 15 minutes at 3000 rpm to get serum sample and then sera were collected in Eppendorf tubes then kept frozen at -20ºc till be analyzed.

4.2 Tissue samples

By the end of the 5^{th} day of the oral dose of Cephradine, five chicks were slaughtered from each group, from all slaughtered chicks, samples of kidney, liver, and breast muscle were collected for estimation of Cephradine residues at 24,48,72,96 hours following the last dose in 15ml falcon tube then kept in a deep freezer at -20ºc till transported in ice box to assay the level of Cephradine residues at Central Lab of Faculty of Veterinary Medicine, Suez Canal University.

5. Analytic procedure

Cephradine concentration was determined in serum and tissue samples by CAMAG HPTLC apparatus which is equipped with: Nanomate4, Linomate 5, ATS4, automatic development chamber (ADC2, AMD2), CAMAG Derivatizer, and TLC scanner4.

6. Chemicals and standard reagents

The purity of all chemicals, standards, and reagents was at least 99% HPLC grade. Methanol **(Carlo Erba, France),** ethyl acetate **(advent, India),** ethanol, ninhydrin reagent, and double-distilled water.

6.1. Standard preparations

Methanol was used to make stock solutions containing 500 µg/ml of Cephradine. Working standard solutions containing 0.5-50µg/ml Cephradine was made by diluting stock solutions with methanol to the appropriate concentration ***(Saleh et al., 2010).***

6.2. Reagent preparation

Ninhydrin 0.5% ethanolic solution: carefully weigh 500mg ninhydrin powder and dissolve in 100ml ethanol.

7. Sample preparation

Sample extraction was described by ***Fabre et al. (1985)*** with slight modification. For assay of tissue samples, kept frozen samples were thawed completely, one gram of tissue (liver, kidney, and breast muscle) were weighted, added in 15ml Falcon tubes contained 2ml of mobile phase then homogenized for at least 3 min by homogenizer and the suspension with solid parts was mixed thoroughly in a vortex, kept in an ultrasonic bath for 15 min then centrifuged at 5000 rpm for 10 min. following filtering through a 0.45µm nylon filter, the collected Supernatant was collected in another screw-capped glass tube and kept frozen till analysis.

For assay of serum samples, one ml of the collected serum sample was mixed with 2 ml of mobile phase (Ethyl acetate, methanol, and double-distilled water 70:50:30) and centrifuged at 3000 rpm for 5 min then the supernatant was collected in another screw-capped glass tube and kept frozen till analysis.

8. High Performance Thin Layer Chromatography

HPTLC will be carried out on 20cm×10cm HPTLC silica gel 60F254 plates (Merck) with a mobile phase of ethyl acetate: methanol: double distilled water as 70:50:30 ratios. All samples and standards were applied to the plates using CAMAG Linomat 5 with dosing syringe 100µL as 6 mm bands with 10.5 mm. Distance between tracks, application x 10mm and 10mm application Y edges of the plate and the application volume will be 2-4 µL for samples and standard. The

HPTLC plates will be developed to a distance of 70 mm in CAMAG Automatic Developing Chamber ADC2 at room temperature (25ºc±5ºc). The development was carried in two -steps (preconditioning with 10ml mobile phase for 15 minutes and development with 25ml mobile phase for 25 minutes). The plates will be developed to a distance of 85mm and dried for 5 minutes by a stream of warm air. the plates will be scanned and examined densitometry at l=5 nm using CAMAG TLC Scanner 4 with slit dimensions of 6×0.30 mm at multiwavelength of 240, 250, and 260nm. The chromatogram is evaluated under white or UV light. All the functions of the scanner were controlled by the software Win CATS. Only the positioning of the objects to be scanned was performed. The plates were immersed in ninhydrin reagent then heated for 10 minutes at 110ºC in an air oven. At this time, a blue to violet spot on pink background corresponding to Cephradine persisted on the plate

9. Statistical analysis

Obtained results were statistically analyzed to show the least significant difference by (T-test (ANOVA test)) at p>0.05 using SPSS version 22 computer program.

Results

Administration of Cephradine 15mg/Kg. B.Wt. at (0.5 gm/ Liter) in drinking water for 5consecutive days either alone or in combination with potassium citrate (1 gm / Liter) in drinking water revealed a wide distribution of Cephradine in tested tissues as shown in tables (1, 2) and figures (1,2,3, 4).

The mean concentrations of Cephradine residue in the group (1) which received Cephradine alone were 1.61±0.35, 1.45±0.19, 4.85±0.48, and 5.95±0.47 µg/gm on the 1st day after the last dosage in serum, breast. Muscle, liver, and kidney respectively. They were 0.03 ±0.01, 0.49±0.07, 1.19±0.12, and 1.87±0.16 µg/gm on the 2nd post the last dosing. On the 3rd day, Cephradine residues were 0.00, 0.20±0.04, 0.39±0.07, and 0.67±0.12 µg/gm while on the 4th day post the last dosage, they were 0.00, 0.00, 0.19±0.08, and 0.31±0.07 in serum, breast muscle, liver, and kidney respectively.

Generally, administration of potassium citrate during the last 2 days of Cephradine treatment in broiler chicks decreased Cephradine concentrations in serum, muscles, and liver of treated chicks, and even resulted in Cephradine disappearance (became under our detection limit) in the tested samples 1-3 days earlier than using Cephradine as mono-therapy.

On the other hand, potassium citrate administration significantly increased Cephradine concentrations in kidney tissues after 1 day of the last dose of Cephradine administration, and then decreased Cephradine residual concentrations in the 2nd, 3rd, and 4th days after the last dose of Cephradine administration, as shown in table (2).

Table (1): *Serum and tissues concentrations of Cephradine broiler chicks (n=5) after repeated oral dose of 15 mg Cephradine/kg body weight/day for 5 consecutive days without potassium citrate.*

Groups	Days post Treatment	Cephradine concentrations (µg/ml or gm)			
		Serum	Breast M.	Liver	Kidney
Cephradine	1	1.61±0.35	1.45±0.19	4.85±0.48	5.95±0.47
	2	0.03±0.01	0.49±0.07	1.19±0.12	1.87±0.16
	3	UDL	0.20±0.04	0.39±0.07	0.67±0.12
	4	UDL	UDL	0.19±0.08	0.31±0.07

UDL= concentration under detection limit

Table (2) *Serum and tissues concentrations of Cephradine broiler chicks (n=5) after a repeated oral dose of 15 mg Cephradine/kg body weight/day with potassium citrate.*

Groups	Days post Treatment	Cephradine concentrations (µg/ml or gm)			
		Serum	Breast M.	liver	Kidney
Cephradine plus potassium citrate	1	0.35±0.09 *	0.67±0.08 *	0.78±0.11 *	7.67±0.60 *
	2	UDL	0.19±0.03 *	0.24±0.06 *	1.45±0.24
	3	UDL	UDL*	UDL*	0.36±0.04 *
	4	UDL	UDL	UDL*	0.10±0.06

UDL= concentration under detection limit

* = Significantly differs from Cephradine administered group at $p \leq 0.05$

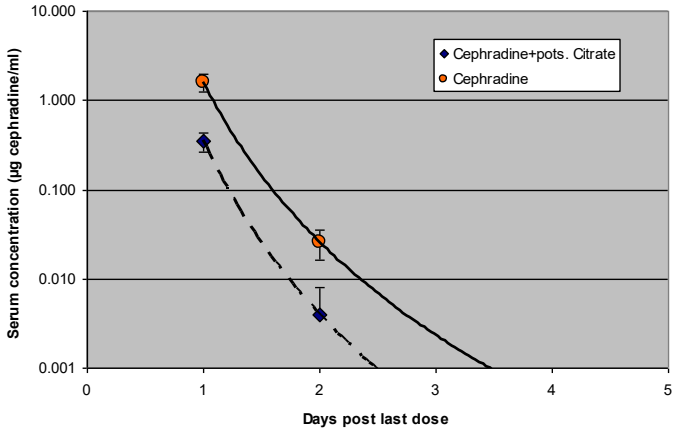

Figure (1): Comparison between serum concentrations (Log) of Cephradine (µg/ ml) versus time (days) after repeated oral doses (15mg/kg) in broiler chicks (n=5) either alone or plus potassium citrate.

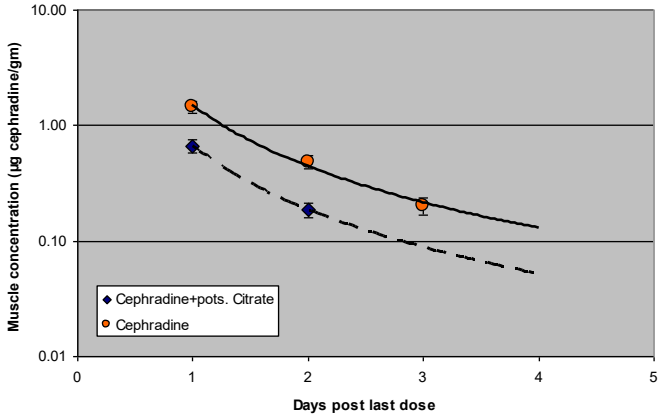

Figure (2): Comparison between Cephradine residual levels (Log) versus time (days) in breast muscles of broilers received repeated oral doses of Cephradine (15mg /kg/day) either alone or plus potassium citrate.

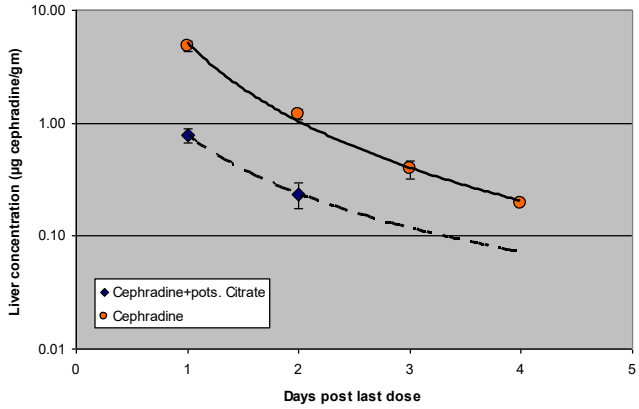

Figure (3): Logarithmic concentrations of Cephradine residues versus time (days) in livers of broilers received repeated oral doses of Cephradine (15mg /kg/day) either alone or with potassium citrate.

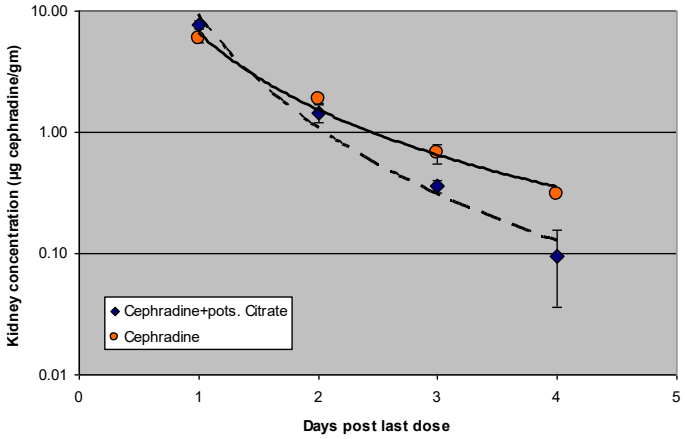

Figure (4): Logarithmic concentrations of Cephradine residues versus time (days) in kidneys of broiler received repeated oral doses of Cephradine (15mg/kg/day) either alone or with potassium citrate.

Discussion

The highest Cephradine concentrations were detected in the kidney and in the liver respectively, which declined exponentially to reach the lowest detectable levels at 4 days post last dose. The lowest concentrations were assayed in the serum and the breast muscle (up to 2 and 3 days

post last dose, respectively). Our results resembled those of ***Weliky et al. (1974)*** who stated that the greatest concentration of Cephradine was measured in the kidney (3.1 mg/Kg) and the liver (1.9 mg/Kg) respectively, as the main route of Cephradine elimination is renal excretion (mainly in unchanged form). Moreover, the authors measured the Cephradine skeletal muscle concentration (1.3 mg/Kg) and found it was less than both kidney and liver. Also, our results match with those obtained by ***El-Gendy et al. (2010)*** who investigated pharmacokinetic aspects of Cephradine following a single dose of 50 mg kg^{-1} B.Wt. was given intravenously, intramuscularly, subcutaneously, and orally in chickens. They recorded the highest drug concentration in the chick's kidneys (1.09 µg/gm) while it was less than one-third of that concentration in the liver (0.29µg/gm). In contrast, ***El-Sayed et al. (2016)*** studied the pharmacokinetics of Cephradine (20 mg/kg B.Wt.) following intravenous and oral administrations in healthy and *Salmonella enteritis* infected broiler chickens. The authors found that the highest Cephradine residual concentrations were in the liver (up to 5 days) and kidney (up to 3 days). In another study, ***Aboubakr et al. (2017)*** investigated the tissue residues of Cephradine after oral administration (20 mg/kg B.Wt. for five days) in healthy and infected broiler chickens. In addition, the authors reported higher Cephradine concentrations in the liver than in the kidney (11.32 and 1.28 µg/g at 3 days post last dose).

The variation between their and our results could be attributed to the difference in Cephradine assaying methods, as we used the HPTLC method while both authors used the microbiological method. The microbiological method detects not only the parent drug (Cephradine) but also detects the active metabolites as well. That could be the reason for the contradiction between their and our results.

When potassium citrate was administered orally to Cephradine-treated chicks, the Cephradine residues disappeared from the breast muscles and liver 1 and 2 days earlier than when the Cephradine was administered alone. in the kidney, the Cephradine residues were still measurable up to 4 days at very low concentrations. That may be because the kidney is the main route of drug excretion ***(Schwinghammer et al., 1990)***.

Hence, our results showed that the co-administration of potassium

citrate reduced Cephradine residues in broiler's edible tissues, consequently, shortened the Cephradine withdrawal period.

Most drugs are filtered through the glomerulus into the renal tubules. If a drug has high lipid-solubility, a large portion will be reabsorbed into the bloodstream through passive diffusion. To speed up renal excretion of the drug, it is worthy to avoid its reabsorption from the renal tubules. This can often be achieved by changing the pH of the urine to force the majority of the drug molecules to be in the ionized form. Hence, the drug will be "trapped" in the urine. Thus, weak acids are excreted more quickly in alkaline urine ***(Katzung, et al., 2012)***. Potassium citrate was found to be a urinary alkalinizing diuretic ***(Suarez et al., 2015)***. That will result in the ionization of acidic drugs, such as Cephradine, preventing their tubular re-absorption and consequently enhancing their renal excretion.

Conclusions

It can be concluded that Cephradine can be detected in the kidney, liver at the highest concentration, with the lowest concentration in serum and breast muscle. Potassium citrate is effective in reducing residues of Cephradine in serum and edible tissues of broiler chicks.

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تأثير سترات البوتاسيوم على بقايا السيفرادين في بدارى التسمين

أجريت الدراسة الحاليه لتقدير بقايا السيفرادين في مصل وأنسجة بداري التسمين بعد تناوله عن طريق الفم لمدة خمسة أيام متتاليه وكذلك الكشف عن فاعلية سترات البوتاسيوم على بقايا السيفرادين في مصل وأنسجة بدارى التسمين. تم استخدام 40 كتكوت عمر 21 يوم من بداري التسمين (هيبرد) في هذه الدراسة بعد تقسيمها الي مجموعتين متماثلتين (كل منهما 20 كتكوت). تم اعطاء السيفرادين (أتوسيف فورت) جرعة فمويه قدرها 15 ملجم / كجم من وزن الجسم مرتين يوميا لمدة خمسة أيام متتالية للمجموعة 1 وتم إعطاؤه مرتين يوميًا لمدة 3 أيام ولكن في اليومين الأخيرين ، تم إعطاء سيفرادين لمدة 12 ساعة وسيترات البوتاسيوم لمدة 12 ساعة أخرى في المجموعة 2. تم قياس متبقيات السيفرادين في مصل الدم والكبد والكلى وعضلات الصدر لدجاج اللاحم عند 24 ، 48 ، 72 ، 96 ساعة بعد آخر جرعة باستخدام اختبار HPTLC. كشفت النتائج عن امتصاص وتوزيع جيد للسيفرادين في الكبد والكلى وعضلات الصدر. وقد أمكن الكشف عن بقايا سيفرادين في مصل الدم حتى اليوم الثاني ، في عضلات الصدر حتى 3 أيام ولكن في الكبد والكلى حتى اليوم الرابع. من ناحية أخرى ، تناول سترات البوتاسيوم مع السيفرادين تسبب في انخفاض كبير في تركيز الدواء في مصل الدم والكبد والكلى وعضلات الصدر. يمكن الاستنتاج أنه يمكن الكشف عن سيفرادين في الكلى والكبد بأعلى تركيز مع أقل تركيز في مصل الدم وعضلات الصدر كما أن سترات البوتاسيوم فعاله في تقليل بقايا سيفرادين في مصل الدم والانسجة.

الكلمات الدالة: سترات البوتاسيوم- السيفرادين- المتبقيات