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EFFECT OF BOILING AND FREEZING ON CIPROFLOXACIN RESIDUES IN CHICKEN TISSUES

(With 2 Tables)

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تأثير الغليان والتجميد على بقايا السبروفلوكساسين فى أنسجة الدجاج

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نظرا للإستخدام الواسع والمكثف للمضادات الحيوية في مزارع الدواجن بغرض زيادة النمو ومقاومة الأمراض مما ينتج عنة وجود الأدوية في لحوم وأعضاء الدواجن عند تناولها لهذا تم إجراء هذة الدراسة بغرض الكشف عن بقايا مركب السيبروفلوكساسين من مجموعة الفلوروكينولون وكذلك تأثير الغليان و التجميد على بقايا هذة الأدوية في أنسجة الدواجن عقب إعطائها الجرعة العلاجية ثم قياس هذة البقايا بواسطة جهاز الفصل الكروماتوجرافي السائل عالي الأداء. تم إستخدام خمسين كتكوت ووضعت تحت نظام غذائي متوازن خالي من أية عقاقير وعند الأسبوع الخامس تم إعطاء الدجاج جرعة علاجية من السيبروفلوكساسين عن طريق الفم ٥ ملجم/كجم من وزن الجسم. و تم ذبح خمس دجاجات عند اليوم (الأول، الثالث، الخامس، السابع والتاسع) وبعد الجرعة الأخيرة تم تقدير بقايا المركب قبل و بعد الغليان عند درجة حرارة ١٠٠ م لمدة نصف ساعة في عضلات (الصدر، الفخذ، الكبد والدهن) كذلك أخذ العينات من خمس دجاجات وفحصها قبل وبعد التجميد عند (-١٨) م اسبوعيا لمدة خمسة أسابيع متتالية. ومن خلال الدراسة اتضح ان اعلى تركيز لمركب السبروفلوكساسين فى الكبد عند اليوم الأول من الجرعة الاخيرة ٢٠,٧٥ ±٠,٧٠ ميكروجرام/جم والذى يتناقص ولم يستدل علية عند اليوم التاسع. ثم تلاة الدهن وكان تركيز المركب ١٨,٠٥±٠,٦٤١ ميكروجرام/جم وكان أقل تركيز في عضلات الفخذ و الكبد (١٤,٩٨± ٠,٧٨، ١٢,٦ ±١,٠٢٥) ميكروجرام/جم. أما بالنسبة لتأثير الغليان فكان تركيز المركب في الكبد ١٦,٣٧±٠,٢٤٠ ميكروجرام/جم عند اليوم الأول وقل تركيزة ولم يستدل علية عند اليوم التاسع. ثم تلاة الدهن فكان تركيز المركب بعد الغليان ١٢,٣٧± ٠,٢٤٠ ميكروجرام/جم و لوحظ أن أقل تركيز في عضلات الصدر والفخذ ٩,٨٧ ± ١,٠٥ ، ٠,٥٣١± ٠,٥٧ بينما أنسجة الكبد الذي حفظت بالتجميد عند -١٨ م لوحظ أن التركيز انخفض بعد الأسبوع الأول إلا أنة لم يستدل علية عند الأسبوع الخامس. أما فى الدهن وعضلات الصدر فلم يستبدل على وجود المضاد الحيوى فى الأسوع الرابع وفى عضلات الفخذ لم يتم اكتشاف أى أثر للمضاد الحيوى فى الأسبوع الثالث. وتم التوصية بعدم الذبح قبل مرور عشر أيام من أخر جرعة حتى يتأكد من عدم وجود أى أثار للمركبات والحصول على لحوم دواجن صالحة للاستهلاك.

مرور عشر أيام من أخر جرعة حتى يتأكد من عدم وجود أى أثار للمركبات والحصول على لحوم دواجن صالحة للاستهلاك.

SUMMARY

Fifty apparently healthy one day old chicks were given orally ciprofloxacin 5 mg/kg b.wt for 5 successive days. Groups of 5 broilers were slaughtered at 1st, 3rd, 5th, 7th and 9th day after administration of the last dose. Muscles (breast and thigh), liver and fat of each broiler group were examined now and after boiling at 100°C for 30 minutes using for determination of ciprofloxacin residue and its, persistence at high temperature. Raw breast, liver and fat samples showed gradual decrease of ciprofloxacin residue whereas a marked decrease was observed in heat treated samples where at 9th day both raw and boiled samples were negative for the drug. Thigh muscles samples proved to be free from any drug residue at 5th day while both raw and boiled samples were free at 7th dose storage. On the other hand, frozen storage (-18°C), broiler, had a marked effect on ciprofloxacin residue in breast, thigh, liver and fat samples. In liver samples the drug failed to be detected at 5th week of storage at -18°C whereas in fat and breast muscle samples, no drug residue could be detected at 4th week. At 3rd week of storage, thigh muscles samples were free from any drug residue The public health significance of ciprofloxacin was discussed.

Key words: *Ciprofloxacin, residues, chicken, boiling, freezing.*

INTRODUCTION

Ciprofloxacin is a new fluoroquinolone antimicrobial agent, with a rapid bactericidal activity against a broad spectrum of bacteria (Sanders *et al.*, 1987).

Nowadays, the intensive farming methods and mass production of poultry are developing more and more, thus the use of antimicrobial as prophylactic means to prevent and control infection and promote growth is increasing rapidly.

The extensive use of these drugs during the whole life time of bird give rise to problem of drug residues. The "residues problem" is the focus of public concern which introduce a serious and novel hazard to the human beings.

These residues comprise the non-altered parent compound as well as metabolites and or conjugates (Haagsma, 1993).

The present study was conducted to detect the possible residues of ciprofloxacin in chicken tissues and the effect of heating and freezing storage on ciprofloxacin residues.

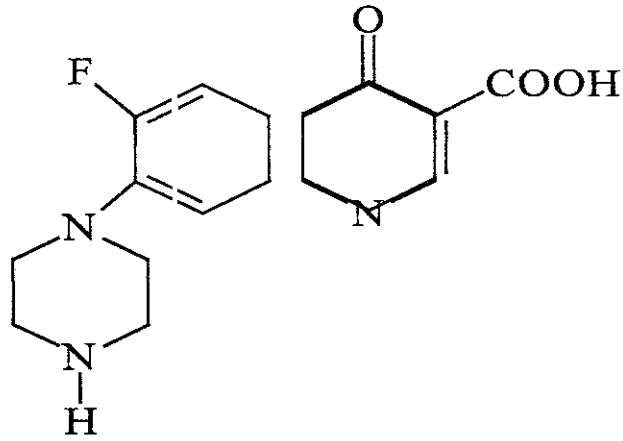
MATERIALS and METHODS

Materials

1- Drug

Ciprofloxacin (ciprotril)®

Ciprofloxacin is a fluroquinolone antibacterial agent with a wide spectrum of activity.



Structural formula of ciprofloxacin (Lebel, 1988)

Chemical formula C_{17} H_{18} FN_3 O_3:

Chemical name: (1-Cyclopropyl-6-fluoro-1,4-dihydro 4-oxo-7-piperazin-1-ylguino line-3-carboxylic acid).

Dose for poultry is 100 ml/ 200 litre of drinking water (5 mg/kg bwt) for 5 days.

2- Experimental chicks:

Fifty apparently healthy, one day old chicks were obtained from El-Salam farm in 10th of Ramadan city. They were fed on a balanced commercial ration free from any medication and water was provided adlibitum. All hygienic measures were adopted as recommended. Temperature was adjusted according to age (Start at 32°C and decreased 2°C each week).

Methods

1- All chicks were vaccinated against Newcastle disease on 6th day of age and against Gumboro disease on 15th day of age.
2- At the 5th week chicks were treated with ciprofloxacin (5 mg/kg body wt) for five successive days for detection of drug residues.
3- five broiler were slaughtered on 1st, 3rd, 5th, 7th and 9th day after the last dose.

   Muscles (breast, thigh) fat and liver samples were examined before and after boiling (100°C for 30 minutes) for detection of ciprofloxacin residues and studying effect of heating on its persistence.
4- Samples from breast, thigh, fat and liver were examined before freezing and kept at -18°C then examined weekly for presence of ciprofloxacin residues. The time elapsed from the onset of freezing till complete disappearance of the residues was recorded.

Detection of ciprofloxacin residues: Ciprofloxacin residues were determined using high performance liquid chromatography (HPLC) Knoure, Inc Germany, according to the method described by Groeneveld and Brouwers (1986). Ciprofloxacin was extracted from samples with dichloromethane and 0.1 M sodium phosphate buffer at pH 7.4 chromatography was performed on an amino-exchange column with the mobile phase and tested using UV defector, UV absorbance and monitored at 278 nm. In to a 10 ml extraction tube of 1 gm of homogenized tissue (breast, thigh, fat and liver) and 1ml of 0.1 M. Phosphate buffer pH 7.4 were added. After adding 5 ml dichloromethane, the tube was stoppered and gently shaked at 100 cycle/min for 10 minutes and centrifuged at 4000 rpm for 10 min at room temperature.

After removing the aqueous layer, the organic layer was transferred into another tube and dried under nitrogen at 50°C.

The residues were dissolved in 1 ml mobile phase using a vortex mixer and sonication, before HPLC analysis depending on concentration, 5-20 ml was infected.

Standard preparation

Ciprofloxacin standard solution was prepared from (100%) drug pure by dissolving a weighed amount of drug in distilled water to make stock solution.

Statistical analysis

It was carried out according to Snedecor and Cochran (1967).

Table 1: Effect of heat treatment (boiling) on tissue concentration (μg/g) of ciprofloxalin at dose of 5 mg/kgb.wt orally for 5 successive days (N = 5)

Time / Tissue	1st		3rd		5th		7th		9th	
	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment	Before heat treatment	After heat treatment
Liver	20.75±0.70 [a]	16.370±0.240[a]	11.76±1.34	5.86±0.561[b]	6.83±0.47	3.610±1.516[a]	1.94±0.021	0.72±0.042	0	0
Fat	18.05±0.641	12.65±0.84[b]	10.87±1.03	4.970±0.27[c]	5.83±0.231	2.941±0.269[d]	1.04±0.721	0.95±0.08	0	0
Breast	14.98±0.78	9.87±1.05[C]	8.72±1.23	2.76±0.68[C]	3.97±0.93	1.43±0.92[d]	0.98±0.13	0.59±0.08[d]	0	0
Thigh	12.6±1.025	5.531±0.57[d]	6.13±0.351	2.53±0.66[d]	0.08±0.01	0	0	0	0	0

Means with the different letters significantly at $p < 0.05$.

Table 2: Effect of frozen storage at -180°C on tissue concentration (μg/g) of ciprofloxacin at dose of 5 mg/kg body weight orally for successive days (N = 5).

Time / Tissue	Before freezing	1st week	2nd week	3rd week	4th week	5th week
Liver	20.75±0.70[a]	8.64±0.630[a]	5.64±0.76[b]	2.13±0.162[b]	0.9±0.051[c]	0
Fat	18.05±0.641[b]	5.64±0.764[b]	4.92±1.31[b]	1.87±0.03[c]	0	0
Breast muscle	14.98±0.78[c]	4.832±0.725[c]	2.87±0.06[b]	0.93±0.121[a]	0	0
Thigh	12.6±1.025[b]	3.942±0.41[c]	1.93±0.71[b]	0	0	0

Means with different letters are significant at $p < 0.05$.

DISCUSSION

Residues of veterinary medicinal products as defined by European union, are pharmacologically active substance (whether active principles, excipients or degradation products) and their metabolites which remain in food stuffs obtained from animals to which the veterinary medicinal product in question has been administered (Van dercreek, 1984).

The potential problem associated with drug residues may be classed in two broad categories. First, aesthetics, consumers call of us don't like the idea of foreign substance being present in food. The second problem is that of potential health risks. There potential health problems include allergic reactions, direct toxic effects and a change in the resistance patterns of bacteria exposed to antibiotics (Weaver., 1992)

In the present study, ciprofloxacin administration orally to chicken at dose of 5mg/kg b.wt for five successive days (Table 1) revealed that the mean highest concentration level of ciprofloxacin was detected in fiver at 1st day from administration of last dose (20.75±0.703 ug/g) and its level showed significant decrease ($p< 0.05$) in the 3rd, 5th, 7th, day (11.76±1.34, 6.83±0.047, 1.94±0.021 respectively) until not detected at 9th day. The effect of heat treatment (Boiling at 100°C for 30 minutes) showed significant decrease (16.370±0.24 ug/g) at 1st day and its level decreased significantly (at $p<0.05$) in 3rd, 5th, 7th (5.86±0.561, 3.610±1.516, 0.72±.042 ug/g respectively) till not detected at 9th day.

The drug concentration level in liver was followed by fat that concentration (18.05±0.641 ug/g) and its level decreased till not detected at 9th day (10.87±1.03, 5.83±0.231, 1.04±0.72 ug/g). The effect of boiling showed a significant decreased till not detected at 9th day (12.65±0.84, 4.970±0.27, 2.941±0.269, 0.95±0.08 ug/g).

Breast muscle concentration level before heat treatment was 14.98±0.78 ug/g which decreased till not detected at 9th day (8.72±1.23, 3.97±0.93, 0.98±0.13 ug/g). The effect of boiling showed significant decreased till not detected at 9th day (9.87±1.05, 2.76±0.68, 1.43±0.92, 0.59±0.08 ug/g), respectively. Lowest ciprofloxacin concentration was detected in thigh muscle 12.6±1.025 ug/g) and decreased till not detected at 5th day from drug administration, (6.13±0.351, 0.08±0.01 ug/g). After heat treatment low concentration (5.531 to 0.57 and 2.53 ± 0.061) of ciprofloxacin could be detected in thigh muscle during 1st and 3rd day and failed to be detected at 5th day.

Forzen storage (-18°C) of liver resulted in gradual decrease in the concentration of ciprofloxacin (8.64±63, 5.64±0.76, 2.13±0.16 and 0.9+0.051 mg/g., receptively) from the 1st till the 4th week of storage until not detected in the 5th week. The same manner was observed for (5.64±0.76, 4.29±1.31 and 1.87±0.03 µ/g, respectively) and breast muscles (4.83±0.72, 2.87±0.06 and 0.93±0.12 µg/g respectively) but at 4th week of storage the drug failed to be detected. As for thigh muscle samples, ciprofloxacin could not be recovered at 3rd week of storage as presented in Table (2).

Our results are in agreement with Elin (1999) who found that the highest concentrations level was in liver followed by fat and muscles where the residues disappeared at 10th day of administration of the last dose. Elinstein *et al.,* (1994) reported that all flouroquinolones are well absorbed after oral administration were flouroquinolones (FQS) are minimally protein bound and widely distributed in body tissue.

Anadon *et al., (1985)* found that when norfloxacin was administrated orally the concentration in breast, fat and liver was 0.05 ug/g on the second day after the end of dosing. Bergeron *et al.*, (1985) reported that the concentration of norfloxacin in kidney parenchyma was 4-12 times of the serum concentration.

Scheer (1987) reported that intravenous injection of baytril showed highest concentration in liver, kidney. Alesling (1990) found that the highest concentration were in liver and breast muscle.

Available literature are lacking any figures concerning the effect of boiling and freezing of ciprafloxalin is fluoroquinolonas are similar to antibiotics in their distribution and activity.

Scheibner (1969) stated the heating of meat at 60°C for 60 minutes had no effect on antibiotic residues, but heating to 90°C minimized to some extent the antibiotic activity. The antibiotic residues completely disappeared immediately if the meat was cooked at cooking temperature for 20 minutes. Chunba (1972) showed that normal methods of cooking destroyed aureomycin and terromycin Katz *et al.* (1972) reported that cooking of broiler tissue and organs containing chlorotetracycline residues converted the residues to isochlorotetracycline which had no known biological activity.

Vandenbrande *et al.* (1972) stated that cold storage of meat reduced the activity of penicillin residue. Hassan (1995) reported that the oral administration of tylosine 25 mg/kg b.wt. twice daily for 5 successive days and boiling of chicken tissues and organs for 30 minutes completely degraded tylosene residues in all tissue samples and at 5th week of

freezing tylosine residues were completely disappeared from liver, fat and breast.

Haagsma (1993) stated that the content of residues of many veterinary drugs decreased not only as a result of food preparing and processing, but also at cooled and frozen storage.

Amer *et al.* (1994) concluded that gentamicine at dose of 6 mg/kg b.wt. intramuscularly daily for 7 successive days disappeared by boiling the muscle samples for 45 minutes and freezing for one week.

Gyhan (1997) found that apramycin sulphate residues in chicken tissues after boiling at 100°C for 45° mintues failed to be detected in liver, kidney, gizzard and fat after 48 hours from the last oral dose and the residues disappeared from breast and thigh muscles, liver, kidney, gizzard and fat after the third week from freezing and disappeared from skin after 2 day from freezing samples.

Pouliques and Morvan (2002) determined the residues of oxolinic acid (OA) and flumequine in freeze-dried salmon muscle with attached skin, using reversed-phase (HP/C). They concluded that the limits of detection were 3.2 and 16 µg/g.

From this study one can conclude that broilers must be slaughtered 10 days after the last dose of treatment to protect the consumer from the public health hazards of antibiotics.

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