

DEPLETION OF OXYTETRACYCLINE FROM CHICKEN TISSUES**Abdelrahman Mostafa^{1*}, Nuha M. A. Agbna², Samia A. Wahab³ and Sania Shaddad⁴**¹PhD Student Faculty of Vet. U of K.²Pharmacologist Faculty of Medicine U of K (Collaborator).³Pharmacologist Vet. Research Admin. Soba (Collaborator).⁴Prof of Pharmacology Faculty of Medicine U of K. (Supervisor).Article Received on
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U of K.**SUMMARY**

Oxytetracycline residue was evaluated in broiler tissues after oral administration of the drug in drinking water. Thirty broiler chickens-4 weeks old age were administered Oxytetracycline in drinking water for five consecutive days at a dose of 200.0 mg/l. Six birds were sacrificed at day 1,3,5,7, and 10 from withdrawal of the drug. Tissue samples from muscle, liver and kidney were collected in plastic containers and stored at -20°C for analysis. The concentration of the drug in different tissues was evaluated using LC-MS/MS. The concentration of the drug

in tissues after one day from withdrawal of treatment was $121.0\mu\text{g/Kg}$ in the muscle, $207.0\mu\text{g/Kg}$ in the liver and $97.6\mu\text{g/Kg}$ in the kidney. From the results of this study in day 1 only the concentration in the muscle ($121\mu\text{g/Kg}$) was above MRL set by EU. On day 3 the concentration in all tissues was below EU MRL. This study indicates that withdrawal period of Oxytetracycline in broiler chickens could be 3 days, which is similar to that recommended by USFDA.

KEYWORD: Depletion, Oxytetracycline, LC-MS/MS, chicken tissues.**INTRODUCTION**

Oxytetracycline is a natural tetracycline compound that is derived from the fungus *Streptomyces rimosus*. Tetracyclines are of great clinical importance because they possess a wide range of antimicrobial activity against aerobic and anaerobic Gram-positive and Gram-negative bacteria. They are also effective against some microorganisms that are resistant to cell-wall-inhibitor antimicrobial agents such as Rickettsia, Mycoplasma pneumonia, Chlamydia spp., Ureplasma and some atypical Mycobacterium and Plasmodium spp.^[1]

Oxytetracycline has wide scope in the treatment of various bacterial, rickettsial and mycoplasmal infections of poultry and other avian species. Despite its broad spectrum of activity, its therapeutic effectiveness is marred by its poor bioavailability by oral route.^[2]

Oxytetracycline has been well tolerated when administered orally or intramuscularly as an aqueous solution. It distributes rapidly and extensively in the body, they enter almost all tissues and body fluids. Oral oxytetracycline absorbed primarily in the upper small intestine and effective blood levels are reached in 2-4 hours. Gastrointestinal absorption can be impaired by sodium bicarbonate, aluminium hydroxide, magnesium hydroxide, iron and calcium salts, milk and milk products. This is because oxytetracycline tends to chelate calcium, it is deposited irreversibly in the growing bones and in dentin and enamel of unerupted teeth of young animals or even the foetus if transplacental passage occurs.

About one third of oxytetracycline is excreted unchanged. Excretion is via the kidneys and gastrointestinal tract; it is also excreted in milk and often higher in mastatic milk. Withdrawal time ranges from 5 days for poultry to 28 days for cattle.^[3]

MATERIALS AND METHOD

Animals

Thirty -day old chicks (ARBER-AKERS) were obtained from a local hatchery in Khartoum and moved to a poultry farm in Soba. All chicks were fed antibiotic-free diet, and received antibiotic-free drinking water ad libitum. They were allowed 4 weeks for acclimatization, the chickens became weighing 1.00 – 1.05 kg.

Instrument and Materials

Analysis was performed on LC-MS/MS

Equipment: Agilent 1260 HPLC, Agilent 6460 LC-MS/MS (Triple Quad. LC-MS), Degasser, Pump, Autosampler, Column oven.

Column used Zorbax SB C18 100X4.6mm 3.5 micrometer from Agilent.

Experiment

The thirty chickens were administered Oxytetracycline (water soluble powder) in drinking water at a concentration of 200.0 mg/l. for 5 consecutive days. Six birds were sacrificed at day 1,3,5,7, and 10 from withdrawal of the drug. Tissue samples from muscle, liver and

kidney were collected in sterile plastic containers and stored at -20°C for analysis by chemical method (LC-MS.MS).

Sample Preparation

After thawing, homogenized tissue ($3 \pm 0.03\text{g}$) was weighed in a 50 ml polypropylene test tube and 200 μl 0.1M EDTA (ethylene diamine tetraacetic acid) was added. Samples were mixed and allowed to stand in dark for 15 min. The antibiotics were extracted from the tissues using 15 ml of 70% methanol and 10 min of shaking. After extraction the samples were centrifuged at 3800 rpm for 5 min. The extracts were then diluted with water by adding 100 μl extract to a polypropylene vial containing 400 μl water. The samples were mixed, filtered on 0.45 μm and injected in the LC-MS.MS. The method of drug extraction and analysis was followed Granelli & Branzell.^[4]

RESULTS

The concentration of the drug in tissues after one day from withdrawal of treatment with 200.0 mg/l of drinking water was 121.0 $\mu\text{g}/\text{Kg}$ in the muscle, 207.0 $\mu\text{g}/\text{Kg}$ in the liver and 97.6 $\mu\text{g}/\text{Kg}$ in the kidney. EU MRL for tetracyclines in chicken tissues are 100 $\mu\text{g}/\text{Kg}$, 300 $\mu\text{g}/\text{Kg}$ and 600 $\mu\text{g}/\text{Kg}$ for muscle, liver and kidney respectively. From the results of this study in day 1 only the concentration in the muscle (121 $\mu\text{g}/\text{Kg}$) was above MRL set by EU. On day 3 the concentration in all tissues was below EUMRL.

Table 1: Tissue residue ($\mu\text{g}/\text{Kg}$) of Oxytetracycline after oral administration of 200.0 mg/l for five consecutive days.

Tissue	Concentrations of Oxytetracycline residues in chicken tissues ($\mu\text{g}/\text{Kg}$)									
	1 day after administration		3 day after administration		5 days after administration		7 days after administration		10 days after administration	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Muscle	121.0	35.05	29.2	3.57	25.0	9.25	11.7	3.63	11.9	0.59
Liver	207.0	21.2	85.4	1.63	82.0	11.43	58.6	2.9	54.0	4.23
Kidney	97.6	8.92	142.2	5.70	101.3	7.08	53.6	9.59	85.9	5.12

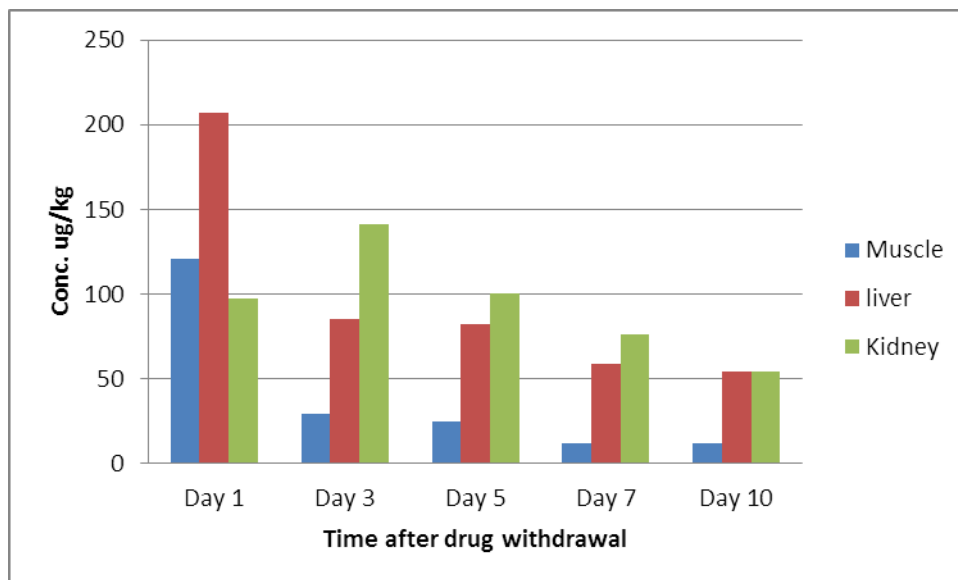


Figure 1: Tissue concentration of residual Oxytetracycline in chickens.

DISCUSSION

The concentrations in muscle and liver were highest on day 1 after withdrawal of medication. The highest concentration in the kidney was found on day 3. The residue levels were significantly higher in liver than in kidney or muscle. This finding were different from those obtained by ALHENDI *et al.*^[5] who found that the highest concentrations of the three tissues liver, kidney and muscle were found in day 1 and the residue levels were higher in kidney than in liver and muscle.

The concentration in the muscle decreased to below MRL on day 3. Which is different from the result obtained by ALHENDI *et al.*^[5] who found that the concentration decreased to below MRL on day 5. The concentrations in the liver and kidney were below the MRL from day 1 onward. These findings were similar in part with that obtained by ALHENDI *et al.*^[5] who found that the concentration in the kidney was below the MRL from day one onward and different from his finding in the liver in which the concentration decreased to below MRL on day 3.

From the study, 3 days withdrawal period could be recommended for Oxytetracycline in broiler chickens which are similar to that recommended by USFDA.

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