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RESEARCH ARTICLE

PHARMACOLOGY

RESIDUAL EFFECT OF CEFTRIAXONE IN BLACK BENGAL GOATS AFTER LONG TERM INTRAMUSCULAR ADMINISTRATION

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ABSTRACT

Ceftriaxone was administered at 25 mg kg^{-1} daily by intramuscular injection for 60 days in goats. Blood, urine and feces samples were collected at every 7 days interval. Residual concentration of ceftriaxone and its metabolite ceftizoxime in blood, urine and feces showed a decreased pattern after 7 days onwards. Ceftriaxone and its metabolite (ceftizoxime) residue were recorded in kidney, lung, liver, spleen, adrenal gland and testis of goat sacrificed on day 60; while only metabolite (ceftizoxime) was identified in heart, fat, ovary, uterus, skin, bone, muscle and brain. Very low level of ceftriaxone and its metabolite (ceftizoxime) residue were detected in various tissues after withdrawal of ceftriaxone for 7 days.

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KEY WORDS

Ceftriaxone, Metabolite ceftizoxime, Goat, Residue.

INTRODUCTION

Ceftriaxone a third generation cephalosporin group antibiotic is active against a wide range of gram positive and gram negative organism[1]. It is highly stable in presence of beta lactamase produced by various microorganisms. Ceftriaxone is used in systemic infection of goats and also in mastitis cause by susceptible bacteria[2].Sometime ceftriaxone is administered for 10 to 20 days to combat the micro-organisms and as a result the antibiotic may be accumulated in important organs of goats which may cause damage to the organs. Besides, human being may consume ceftriaxone contaminated chivon resulting in public health hazards. Chronic exposure of ceftriaxone in goats and their residual status is scarcely available in literature. Hence the present study was undertaken to study residual status after intramuscular administration of ceftriaxone once daily for 60 days at 25 mg kg^{-1} body weight in goats.

MATERIALS AND METHODS

Drug: Ceftriaxone Sodium (analytical grade, purity ≥ 90%) was used as the test chemical obtained from Leben Laboratoreis Pvt. Ltd. Mumbai, India and Ceftizoxime Sodium (analytical grade, purity ≥ 90%) was obtained from Glaxo Smithkline pharmaceuticals Ltd., Nasik, India. All other chemicals used in this study were obtained from E. Merck (India) and Sigma Chemcials Co.; USA.

Animal Treatment: Clinically healthy Black Bengal male and female adult goats (1-1½ year age) weighing between 12-14 kg were used in this experiment. They were caged individually in custom made stainless steel metabolic cages (48 in. × 48 in. × 3 in.). The animals were stall-fed and water was provided *ad-libitum*. The composition of feed was 2 part wheat husk, 1 part crushed maize, 1 part crushed gram and 2 part green. The temperature of the animal room was maintained at 22 ± 3^0C and provided with artificial lighting facilities. Before starting the experiment, the animals were dewormed once with a mixture of albendazole and rafoxanide (Vetalben-R, Indian Immunologicals) at the dose rate of 7.5 mg kg^{-1} body weight. After 21 days of deworming, the animals were acclimatized in experimental environment for 7 days[3]. Institutional Animal Ethics Committee approved experimental protocol before starting the experiment.

Twelve goats of either sex were divided into two equal groups each containing six animals. Group I was considered as control and received the vehicle distilled water only as intramuscular administration once daily for 60 days. Group II received ceftriaxone at 25 mg kg^{-1} by intramuscular injection once daily for 60 days. To study residue of ceftriaxone and its metabolite, blood, urine and feces samples were collected at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days. Concentration of ceftriaxone and its active metabolite (ceftizoxime), was estimated from blood, urine and feces. Three animals of each group were sacrificed on day 60 after last dose administration whilst the rest animals were sacrificed after 7 days of last dosing that is on day 67. Tissue concentration of ceftriaxone and its metabolite (ceftizoxime) were also carried out.

Analysis of ceftriaxone and its metabolite.

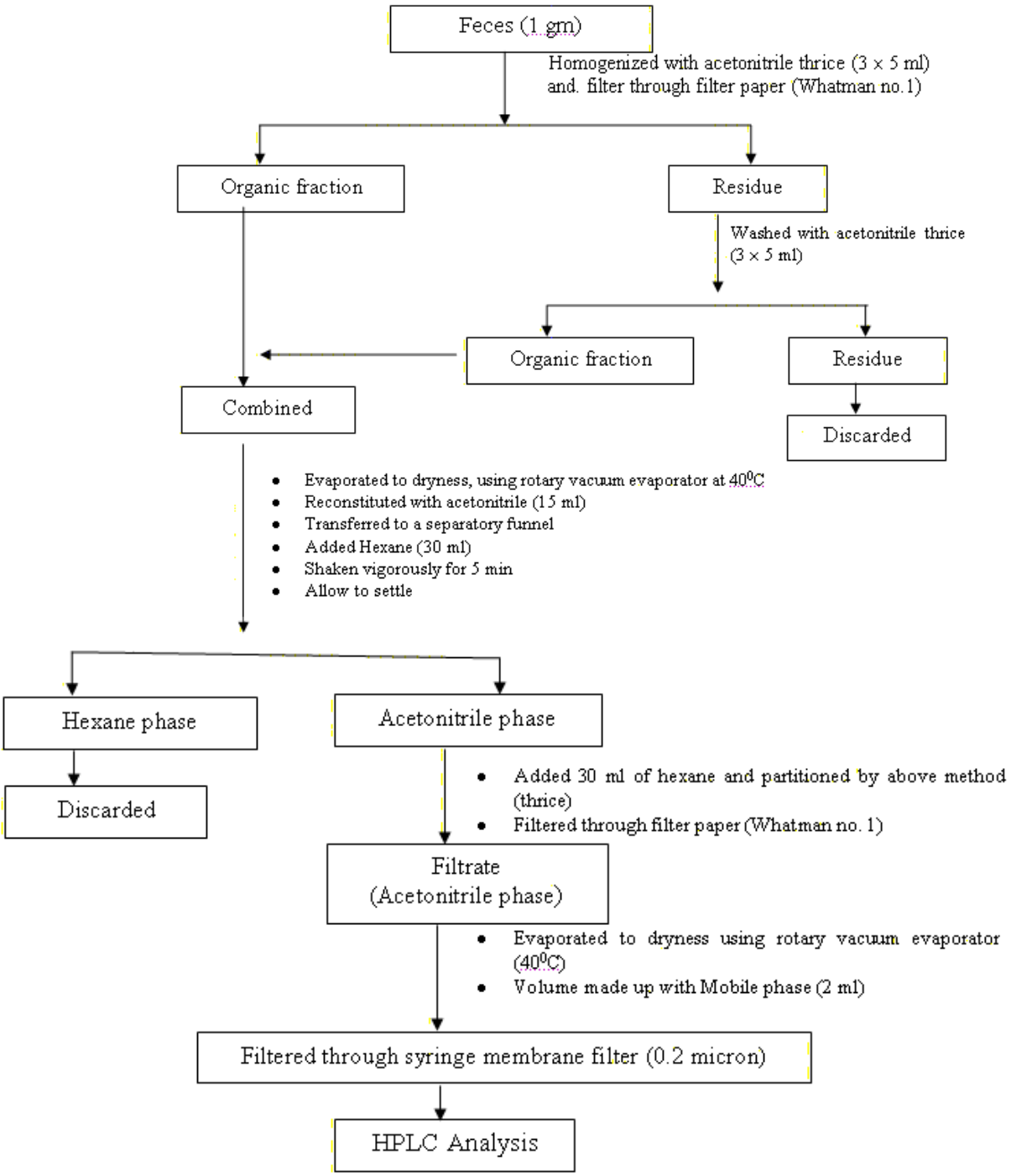
***Blood*:** Blood of individual goat collected in a fixed time after thirty minutes of ceftriaxone administration in a heparinized tube and separate plasma after centrifugation at 2000 rpm for 20 minute. The concentration of ceftriaxone and metabolite (ceftizoxime) in blood was estimated by modified method of Sar et al[4]. To a centrifuge tube containing 1 ml of plasma and 2 ml of acetonitrile was shaken vigorously for 1 min. Then whole aliquot was centrifuged at 5000 rpm for 20 min. The supernatant was collected after filtration (Whatman No. 1). The residue of the test tube washed thrice with actonitrile (3×5 ml) and each time the supernatant was passed through filter paper (Whatman No.1) after centrifugation at 5000 rpm for 20 min. The total filtrate was then evaporated to dryness by using rotary vacuum evaporator. The dried residue was dissolved in 2 ml mobile phase for subsequent analysis by HPLC.

Feces and Urine: Feces and urine of individual goat were collected for 24 hours at every 7 days interval. The excretions were weighed or measured and stored at – 20^0C prior to extraction. The method of extraction of ceftriaxone and its metabolite (ceftizoxime) from feces and urine was carried out following method of Datta *et al*.[5] and presented in scheme 1 and 2 respectively.

Tissue: Three animals of each group were slaughtered on day 60, whilst the rest animals were sacrificed after seven days of last dosing that is on day 67. Samples of liver, kidney, lungs, brain, heart, spleen, adrenal gland, thigh muscle, omental fat, ovary, uterus, testis, skin and bone were taken, weighed, chopped and stored at – 20^0C prior extraction (scheme 3). The same procedure of extraction and quantification was followed for crushed bone and skin except the homogenization step (scheme 3).

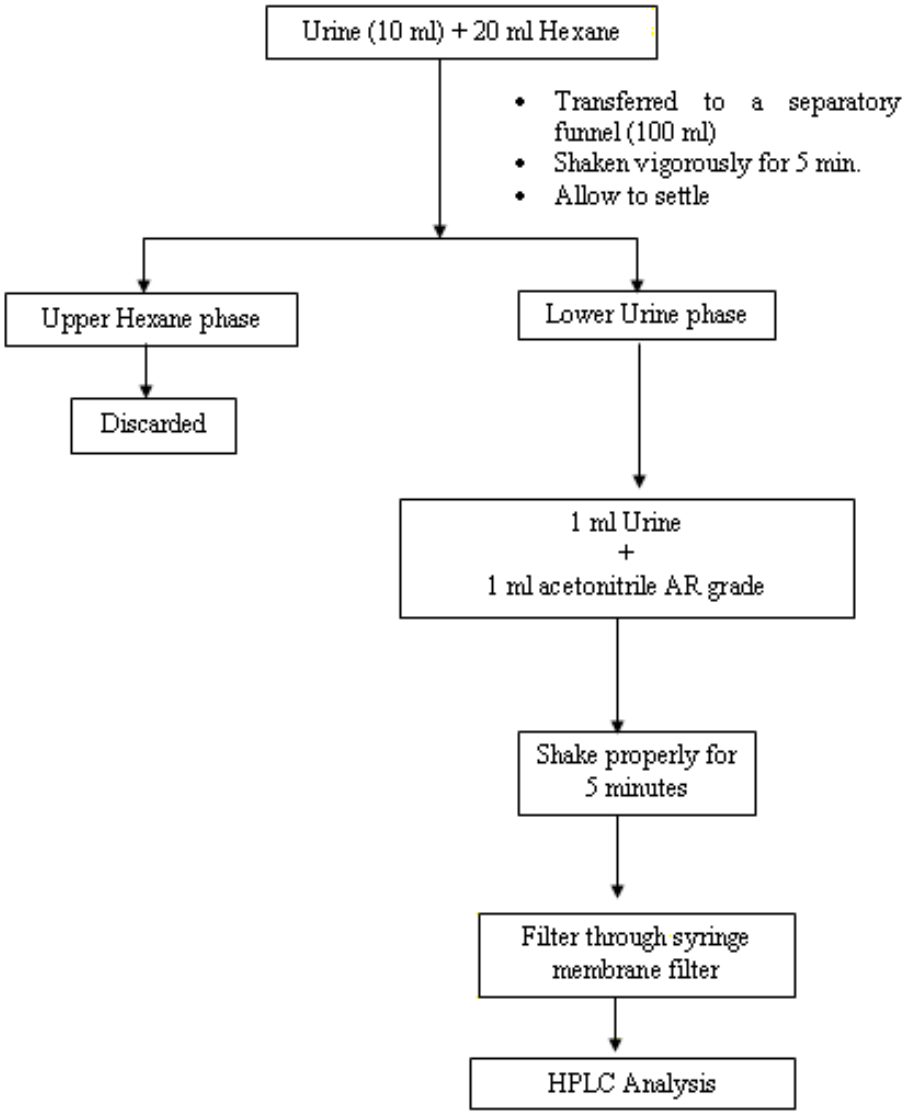
Condition of HPLC: Shimadzu LC-20AT liquid chromatograph coupled with UV photo-diode array (PDA) detector attached with computer. SPDMXA 10 version Software was used for the analysis of cetriaxone and its metabolite ceftizoxime. The operational parameters were as follows: Mobile phase; Glacial acetic acid (5 part), HPLC grade water (75 part) and HPLC grade acetonitrile (20 part) and pH of the mixture was adjust to 2.1. The mixture was then subjected to membrane filtration and degassed. Flow rate was 1ml min^{-1}; Column used; reversed phase C_{18} [5 µ Luna C18 (2); 250 × 4.6 mm (RP)]. Wave length was 254 nm.

A stock solution of 100 ppm of ceftriaxone and ceftizoxime (analytical grade, purity ≥ 90%) was prepared in distilled water as standard. The retention time of ceftriaxone and ceftizoxime was 3.584 min. and 4.931 min respectively (Fig 1). The retention time of the parent compound occurring in blood, tissue, urine and feces was compared with that of the external standard, and the data were recorded in computer SPDMXA 10 software.



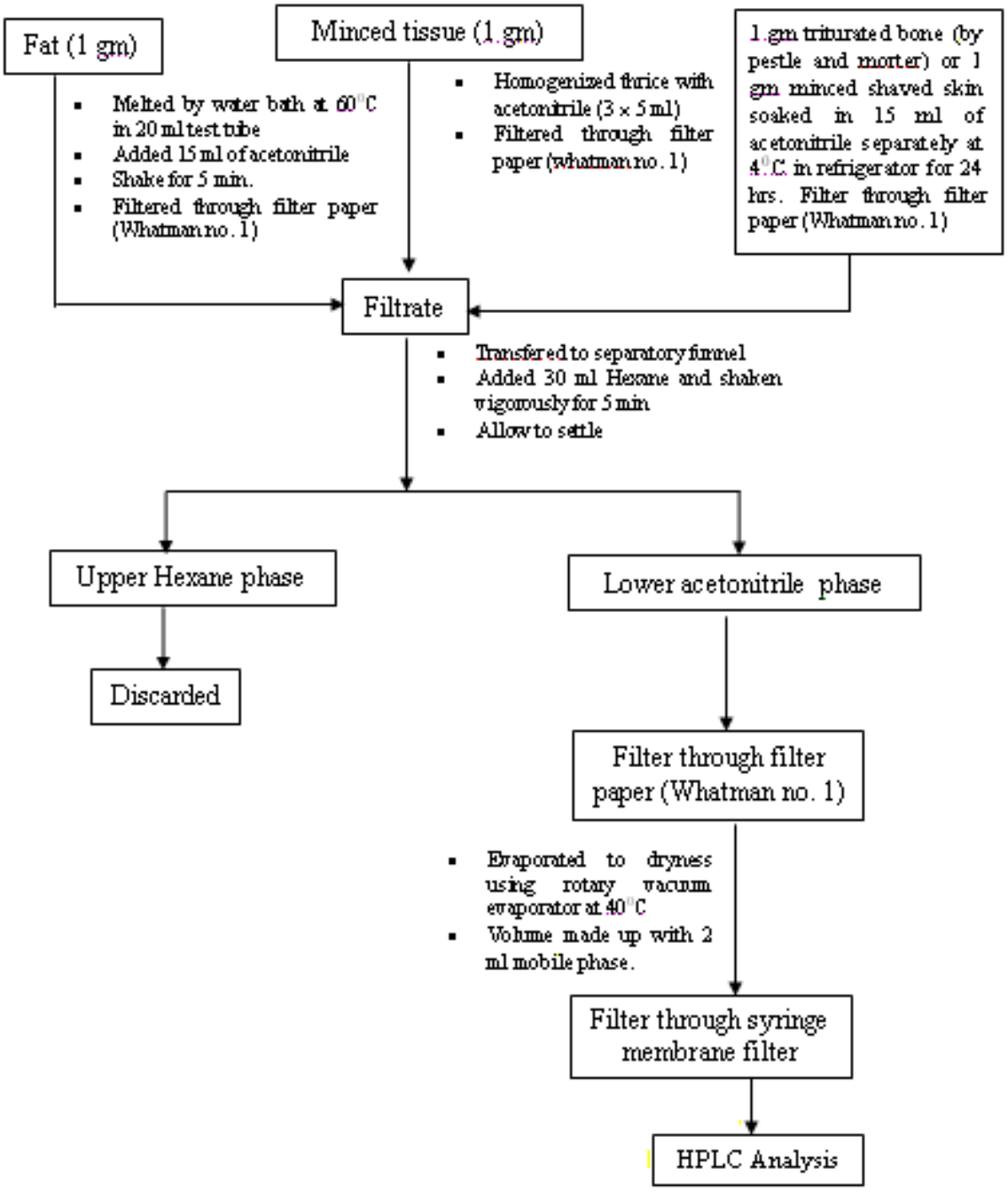
Scheme 1

Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat feces.



Scheme 2

Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat urine.



Scheme 3

Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat tissues

Recovery: The recoveries of ceftriaxone and its metabolite ceftizoxime were estimated by fortifying different substrates with known quantities to give final concentration of 100, 50, 25 and 10 ppm for blood and urine; and 20, 10 and 1ppm for feces and tissue. In plasma, urine, feces and tissue, the limit of detection for ceftriaxone and ceftizoxime were 0.05, 0.03, 0.05, 0.03 and 0.04, 0.03, 0.05, 0.03 ppm respectively. The percentage of recovery from different substrate varied from 85.3 to 90.4.

***Statistical analysis of data*:** The results were expressed as Mean ± standard error (SE). The data were analyzed statistically using one way ANOVA in SPSS 10.0 version of software.

RESULTS AND DISCUSSION

In this present study concentration of ceftriaxone and its metabolite (ceftizoxime) in plasma were decreased significantly after day 14 onwards in test group (Fig.2). In urine and feces both ceftriaxone and its metaboltie (ceftizoxime) concentration decreased significantly after 7 days onward in experimental group (Fig.3 and 4). Ceftriaxone and its metabolite (ceftizoxime) were widely distributed in all organs and tissues. Concentration of ceftriaxone, were detected in kidney, lung, liver, spleen, adrenal gland and testis where as ceftizoxime was identified in brain, fat, bone, skin, heart, ovary, uterus and muscle only.

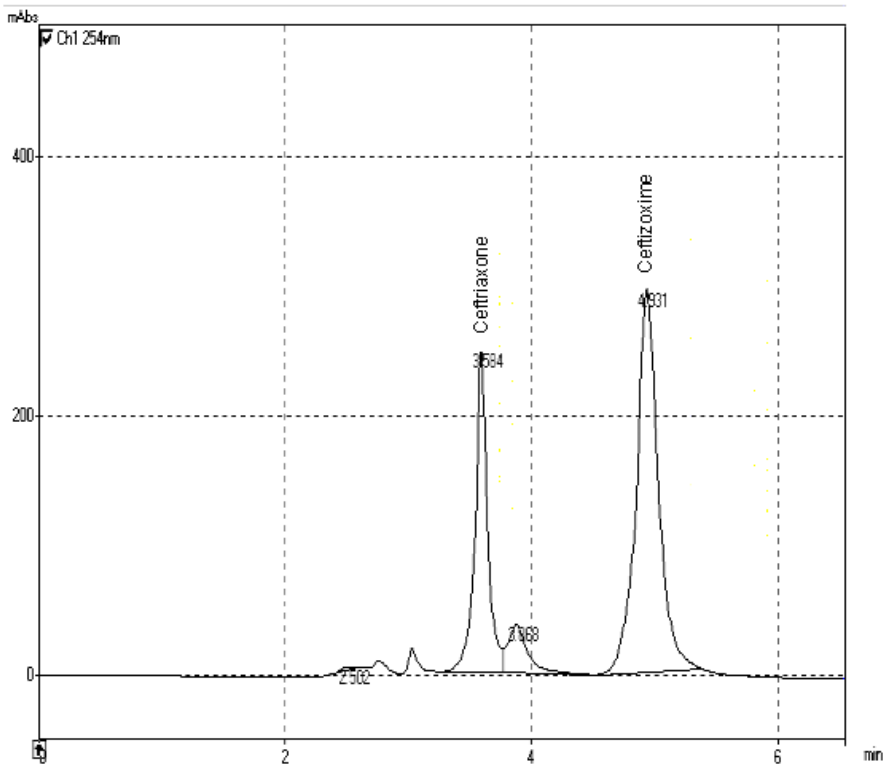


Fig 1

Chromatograph of standard ceftriaxone and ceftizoxime of 50 µg ml^{-1} in distilled water.

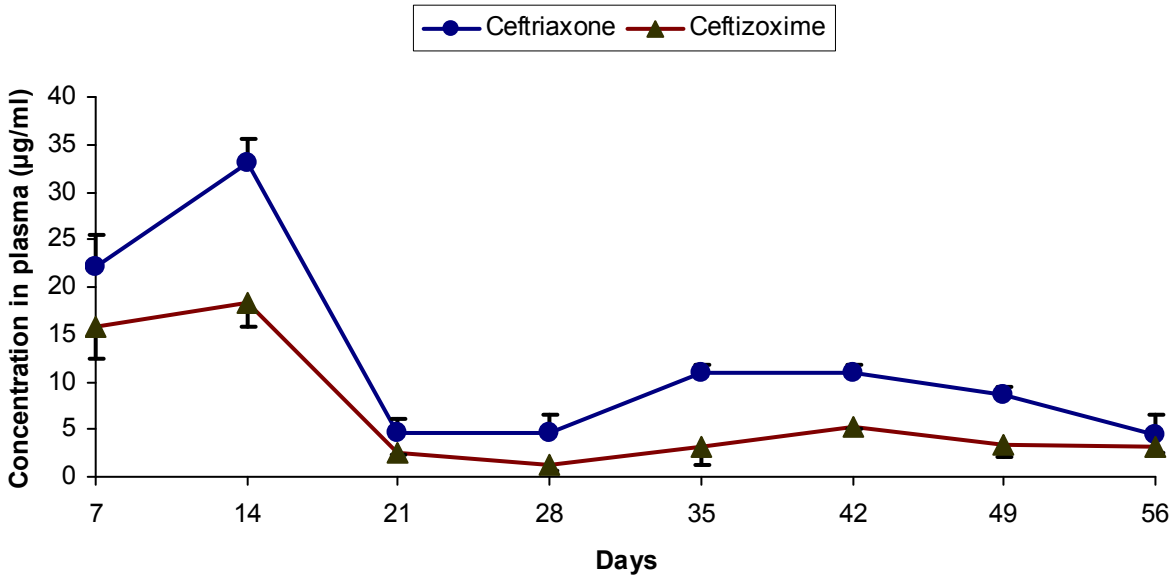


Fig 2

Plasma concentration of ceftriaxone and its metabolite (ceftizoxime) µg ml^{-1} after daily intramuscular administration of ceftriaxone at 25 mg kg^{-1} for 60 days.

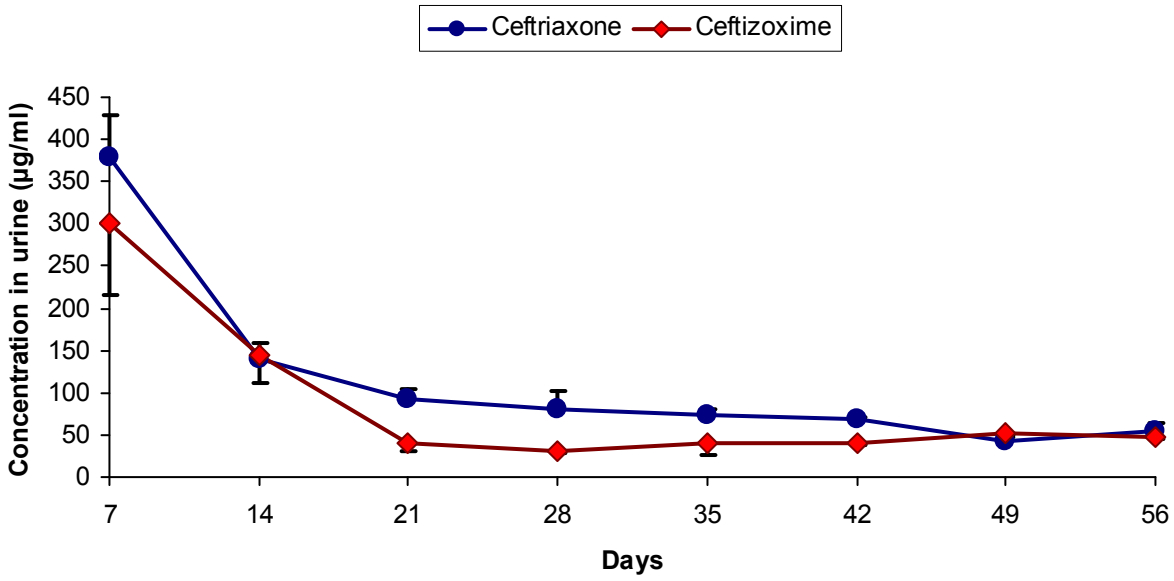


Fig 3

Urine concentration of ceftriaxone and its metabolite (ceftizoxime) µg ml^{-1} after daily intramuscular administration of ceftriaxone at 25 mg kg^{-1} for 60 days.

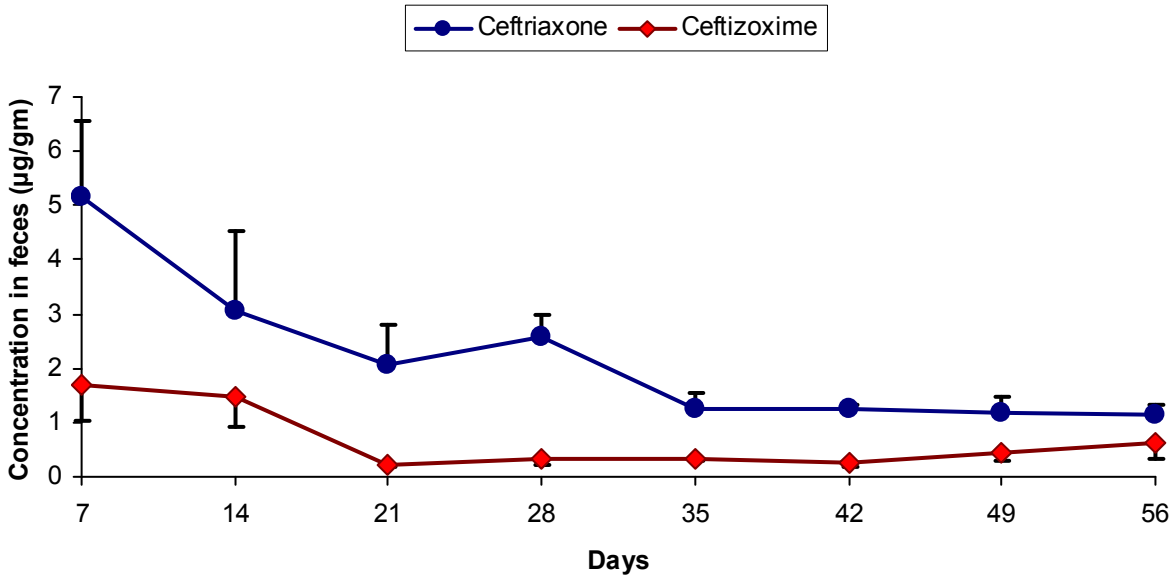


Fig 4

Feces concentration of ceftriaxone and its metabolite (ceftizoxime) µg gm^{-1} after consecutive daily intramuscular administration of ceftriaxone at 25 mg kg^{-1} for 60 days.

Animals sacrificed at day 67 after withdrawal of ceftriaxone administration for 7 days contain significantly low residue of ceftriaxone and its metabolite (ceftizoxime) in tissues. (Table 1)

Table 1

Tissue concentration of Ceftriaxone (μg gm^{-1}) and its metabolite Ceftizoxime (μg gm^{-1}) in goats sacrificed on day 60 and day 67 following consecutive once daily intramuscular administration at 25 mg kg^{-1} dose level for 60 days.

Tissues	Sacrificed on day 60		Sacrificed on day 67	
	Ceftriaxone	Ceftizoxime	Ceftriaxone	Ceftizoxime
Kidney	2.66± 0.52	1.22 ± 0.42	0.29 ± 0.03	0.16 ± 0.02
Liver	0.26 ± 0.04	0.64 ± 0.09	BDL	0.27 ± 0.03
Lung	1.28 ± 0.80	1.04 ± 0.14	0.58 ± 0.06	0.36± 0.06
Heart	BDL	1.33 ± 0.08	BDL	0.06± 0.01
Muscle	BDL	1.32 ± 0.15	BDL	0.08± 0.01
Skin	BDL	1.17 ± 0.09	BDL	0.07 ± 0.01
Bone	BDL	1.14 ± 0.09	BDL	BDL
Spleen	0.06 ± 0.004	0.19 ± 0.02	BDL	0.06 ± 0.01
Fat	BDL	0.83 ± 0.17	BDL	0.14 ± 0.02
Brain	BDL	3.88 ± 0.19	BDL	0.07 ± 0.01

Adrenal gland	0.40 ± 0.07	0.21 ± 0.02	0.06 ± 0.01	0.04 ± 0.007
Testis	0.43 ± 0.15	0.38± 0.12	0.14 ± 0.03	0.13 ± 0.02
Ovary	BDL	1.18 ± 0.17	BDL	BDL
Uterus	BDL	0.33 ± 0.08	BDL	BDL

Abbreviation: BDL, bellow detection limit.

Recovery study showed decreased concentration of ceftriaxone and its metabolite (ceftizoxime) after day 7 in urine and feces and in blood after day 14 onwards. Ceftriaxone undergoes hydrolysis to form active metabolite ceftizoxime[4]. Further, ceftriaxone, increased cytochrome P_{450} content in liver microsome resulting in induction of mixed function oxidase system and therefore, microsomal hydrolysis was inducted due to continued administration of ceftriaxone resulting in increase level of metabolite (ceftizoxime) and consequent decreased level of ceftriaxone[4]. But the concentration of ceftizoxime was also decreased in the present study. Ceftizoxime is an active metabolite of ceftriaxone in goats[4] which may undergo biotransformation in liver to form inactive metabolite resulting in decrease concentration in blood. But the metabolism study of ceftizoxime in goat has not been carried out in the present study. Therefore, further work on metabolism of ceftizoxime is warranted to find out mechanism of decreased level in blood of goats. Ceftriaxone was retained in various tissues up to 5 to 11.6 hour in fowl and rabbit after single intravenous administration in low concentration[6], but in this study it was observed that continuous intramuscular administration cause accumulation of metabolite in most of different tissues instead of parent compound.

CONCLUSION

Therefore it may be concluded from the above research work that administration of ceftriaxone has no significant adverse residual effect in goats and the tissues are devoid of residue after withdrawal period of 7 days.

ACKNOWLEDGEMENT

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