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

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Summary

Ceftriaxone was administered at 50 mg kg^{-1} consecutive once daily by intramuscular injection for 60 days to goats. Blood, urine and feces samples were collected at every 7 days interval. Increased level of AST, ALT, BUN and CRT after 21, 42, 21 and 42 days onward were observed. Ceftriaxone did not alter humoral immunity and also antioxidant status of goats after long term administration. Residual concentration of ceftriaxone and its metabolite ceftizoxime in blood, urine and feces showed a decreased pattern after 7 days onwards. High concentration of ceftriaxone and its metabolite (ceftizoxime) was recorded in kidney, lung and liver of goat sacrificed on day 60 while only metabolite (ceftizoxime) was identified in skin, bone muscle and brain. Low level of ceftriaxone and its metabolite (ceftizoxime) were detected in various tissues after withdrawal of ceftriaxone for 7 days. Histopathology of liver showed mild changes.

Key words: Ceftriaxone, Metabolite ceftizoxime, Goats, Histopathology.

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Introduction

Ceftriaxone a third generation cephalosporin group antibiotic is active against a wide range of gram positive and gram negative organism. 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. 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 caused 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 effect on blood biochemical, residual, immunological and antioxidant status is scarcely available in literature. Hence the present study was undertaken to study blood biochemical, residual, immunological, histopathological and antioxidant status after consecutive once daily intramuscular administration of ceftriaxone for 60 days at 50 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 adlibitum. 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. Institution Animal Ethics Committee approved experimental protocol before starting the experiment.

Twenty goats of either sex were divided into two equal groups each containing ten animals. Group I was considered as control and received the vehicle distilled water only as intramuscular administration consecutive once daily for 60 days. Group II received ceftriaxone at 50 mg kg^{-1} by intramuscular injection consecutive once daily for 60 days. To study long term effect 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), biochemical and I_gG level were estimated from blood, while concentration of ceftriaxone and its active metabolite (ceftizoxime) were determined from urine and faces. Five animals of each group were slaughter on day 60 after last dose administration whilist the rest animals were sacrificed after 7 days of last dosing that is on day 67. Tissue concentration of ceftriaxone and its metabolite ceftizoxime, antioxidant status and histopathology were also carried out.

Analytical procedure:

Analysis of ceftriaxone and its metabolite

Blood -The concentration of ceftriaxone and metabolite (ceftizoxime) in blood was estimated by modified method of Sar *et al.* (2006) [8]. To a centrifuge tubes containing 1 ml of plasma and 1 ml of acetonitrile which was shaken vigorously for 1 min. Then whole aliquot was centrifuged at 5000 rpm for 20 min. The supernatant was collected after passing through a filter paper (Whatman No. 1). The precipitate 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 after keeping in metabolic cage 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 developed by modified methods of sar et al (2006) and it is presented in Fig 1 and 2 respectively

Tissue- Five animals of each group were slaughtered on day sixty, whilst the rest animals were sacrificed after seven days of last dosing that is on day sixty seven. 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 (Fig 3). The same procedure of extraction and quantification was followed for crushed bone and skin except for the homogenization step.

Histopathology- For histopathological examination a portion of liver, kidney, heart, spleen and lung specimens from the control and experimental groups of goats was removed, fixed in formalsaline (10%) processed by using standard paraffin embedding stained with hematoxylin and eosin, and then examined under a microscope [4].

Condition of HPLC- Shimaduz LC-20AT liquid chromatograph coupled with UV photo Diode Array detector attached with computer SPDMXA 10. 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 2.1, the mixture was subjected to membrane filtration and degassed; flow rate was 1ml min^{-1}, column used was reversed phase C_{18} [5 µ Luna C18 (2); 250 × 4.6 mm (RP)]; and Hamilton Microlitre syringe (25 µL) was used; Wave length was 254 nm.

Validation procedure- 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 4). 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.

Fig. 1: Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat feces.

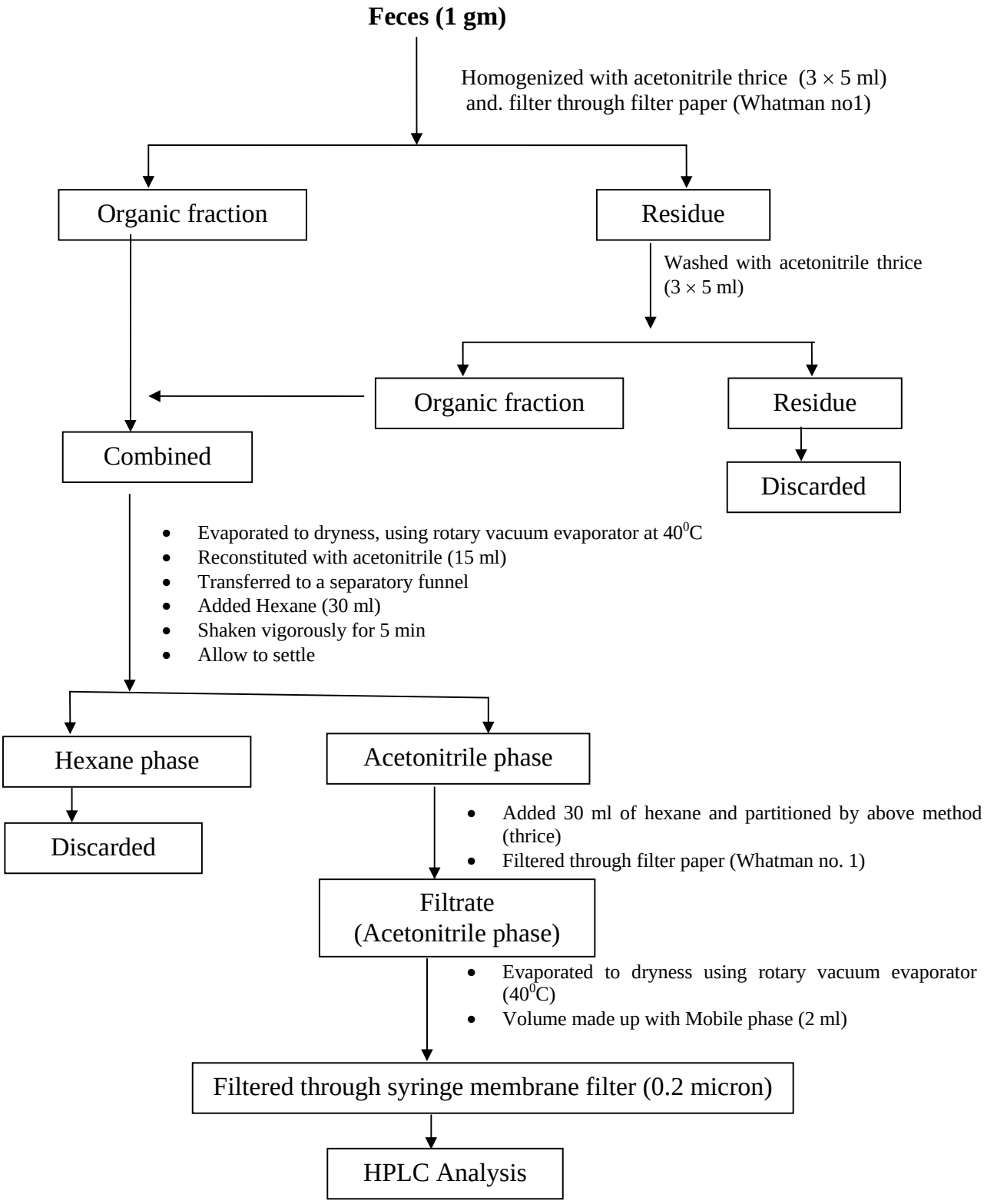


Fig. 2: Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat urine.

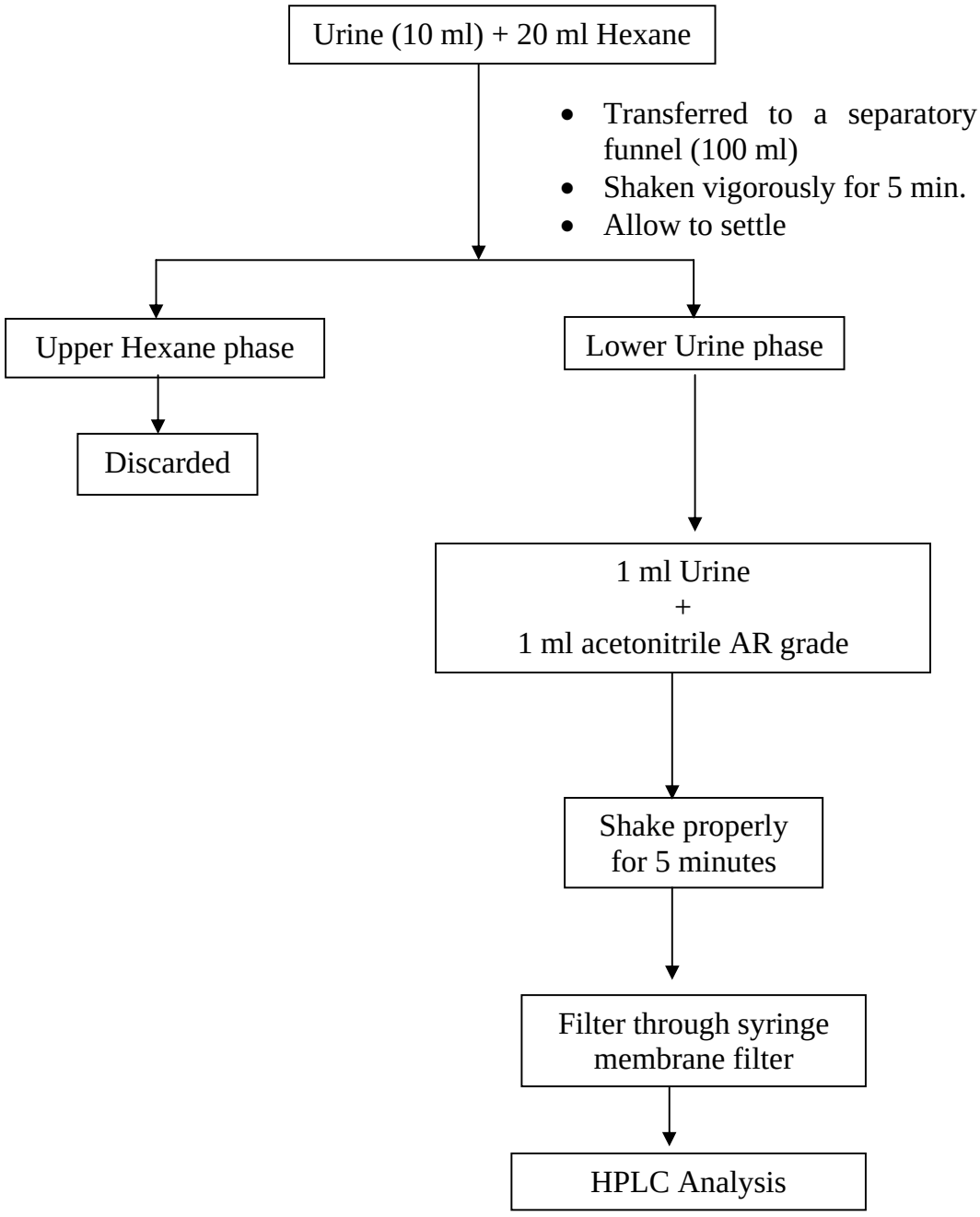


Fig. 3: Extraction of ceftriaxone and its metabolite (ceftizoxime) from goat tissues

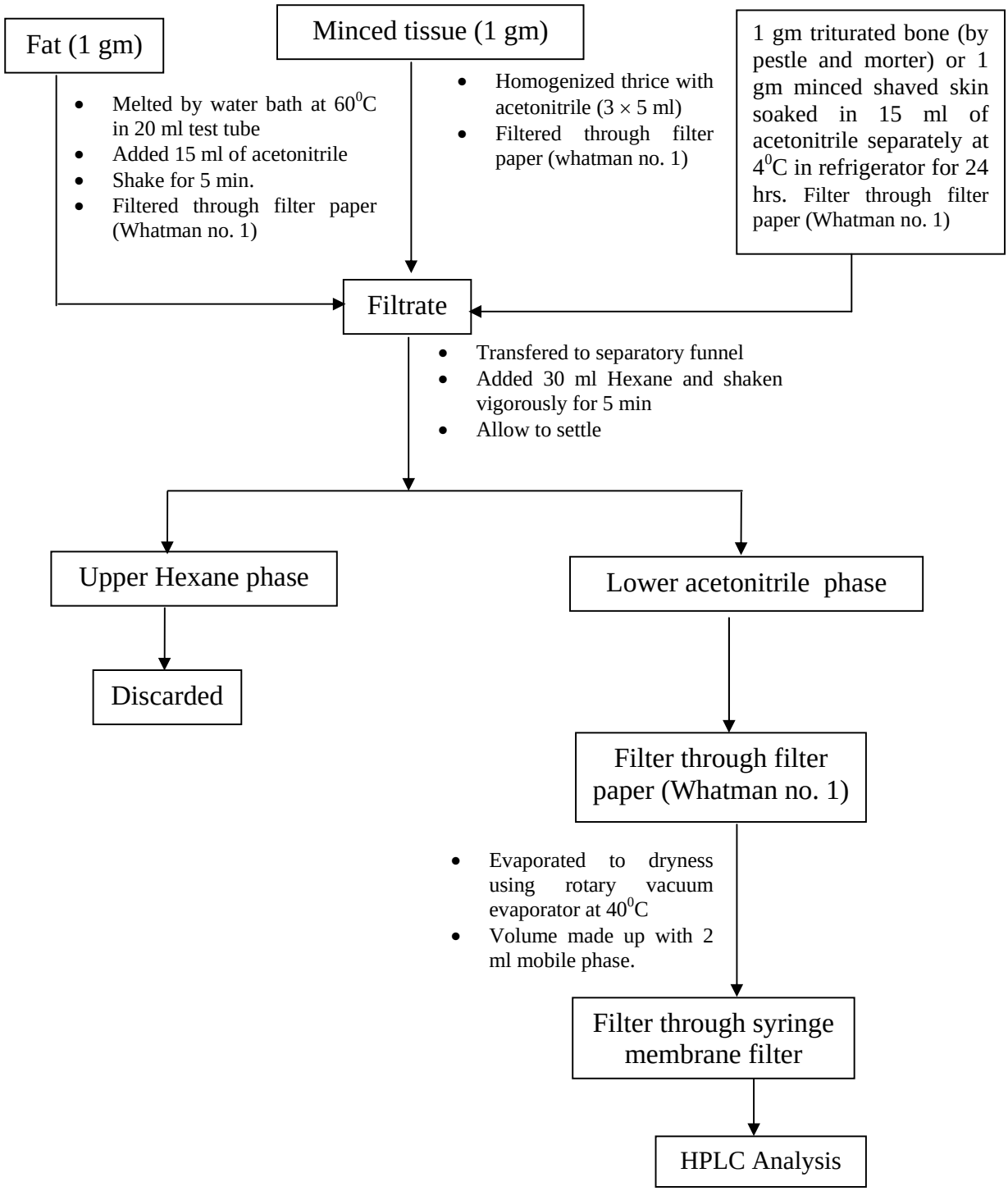
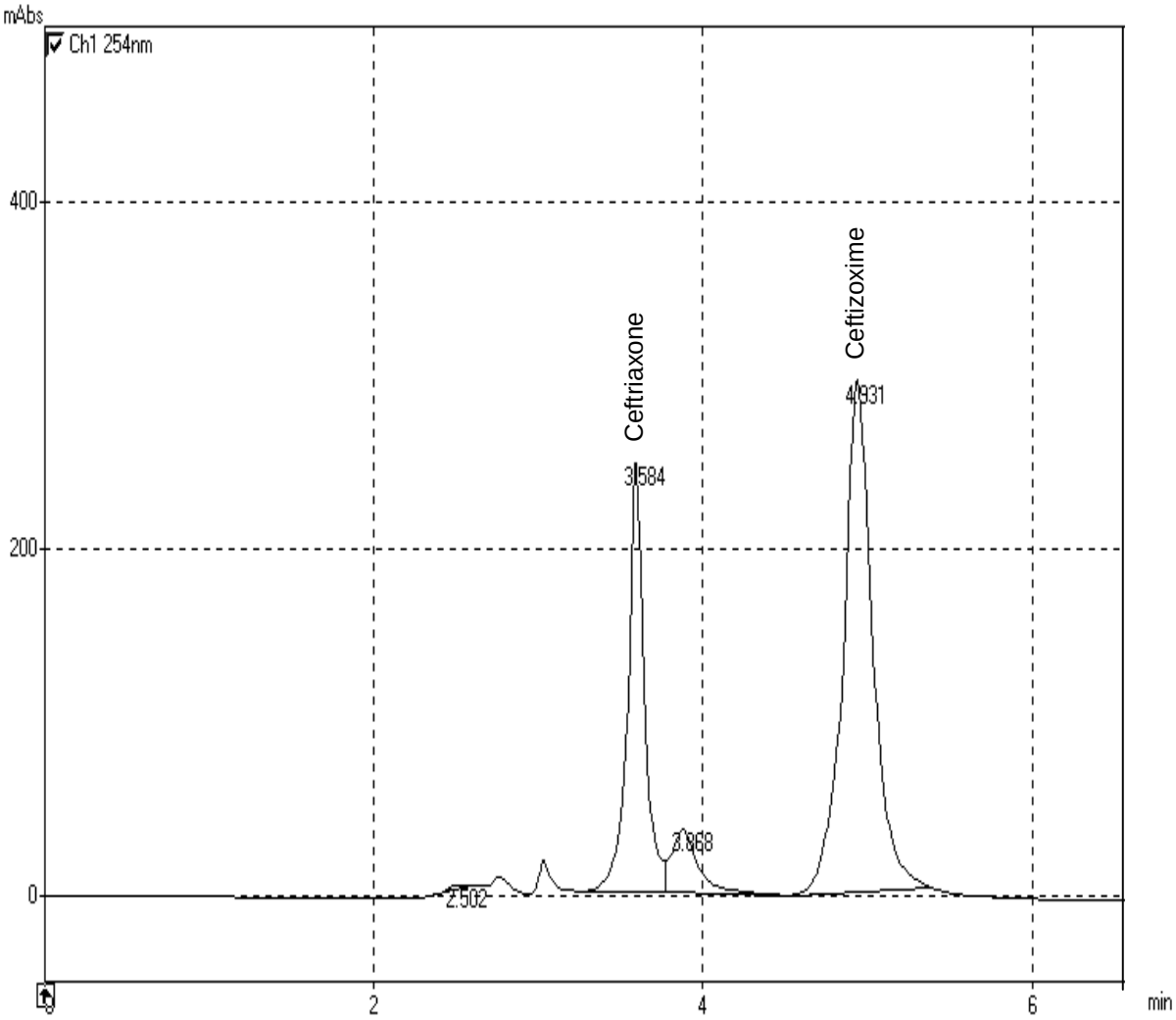


Fig :4 **Chromatograph of Ceftriaxone and Ceftizoxime standard**



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. The limit of detection for ceftriaxone and ceftizoxime were 0.03 and 0.02 ppm respectively. The percentage of recovery from different substrate varied from 85.3 to 90.4.

Blood biochemistry- Blood samples (7 ml) were collected from Jugular vein of each animal of group- I and II at '0' (before administration of ceftriaxone), 7, 14, 21, 28, 35, 42, 49 and 56 day of treatment. Out of 7 ml of collected blood 0.5ml of blood collected in sodium fluoride for glucose estimation [7], 3 ml of blood was allowed to clot for separation of serum and stored in – 20^0C for further use. From collected serum, estimation of protein by Biuret method [10], AST and ALT[11], and immunoglobulin G (I_gG){Sandwitch ELISA method}[3].The ELISA plate was read at 450 nm against blank by using BIORAD 680 microplate reader coupled with computer] were carried out. Rest 3.5 ml of blood was kept into heparinized tube for plasma separation by centrifugation at 3000 rpm for 20 min. and from plasma, plasma urea nitrogen [10], plasma creatinine level [9] and residue of ceftriaxone and its metabolite ceftizoxime were estimated.

Tissue Biochemistry- A part of liver tissue was minced with stainless steel blade, washed in chilled distilled water by using tissue homogenizer (Remi RQ 127A). The crude homogenate (10%) portion of homogenate required amount of chilled distilled water was added in the ratio of 1:1, So that final concentration of homogenate would be 5%. The tissue homogenate was centrifuged in refrigerated centrifuge (Remi C-24) at 4^0C at 6000 rpm for 20 min. The supernatant was utilized for the estimation of catalase [1].

A portion of tissue homogenate (10%) was utilized for estimation of super oxide dismutase[5], Lipid peroxidation [6] ,and reduced glutathione [2].

Statistical analysis of data- The results were expressed as Mean ± standard error (SE). The data were analysed statistically using general linear model with univariate data in SPSS 10.0 version of software.

Results

Recovery of ceftriaxone and its metabolite ceftizoxime

Blood: The concentration of ceftriaxone and its metabolite (ceftizoxime) in plasma were decreased after day 14 onwards in test group. (Fig.5)

Urine and feces- In urine and feces both ceftriaxone and its metaboltie (ceftizoxime) concentration decreased after 7 days onward in experimental group. (Fig.6 and 7)

Fig. 5: Plasma concentration of ceftriaxone and its metabolite (ceftizoxime) µg ml $^{-1}$ after consecutive daily intramuscular administration of ceftriaxone at 50 mg kg $^{-1}$ for 60 days.

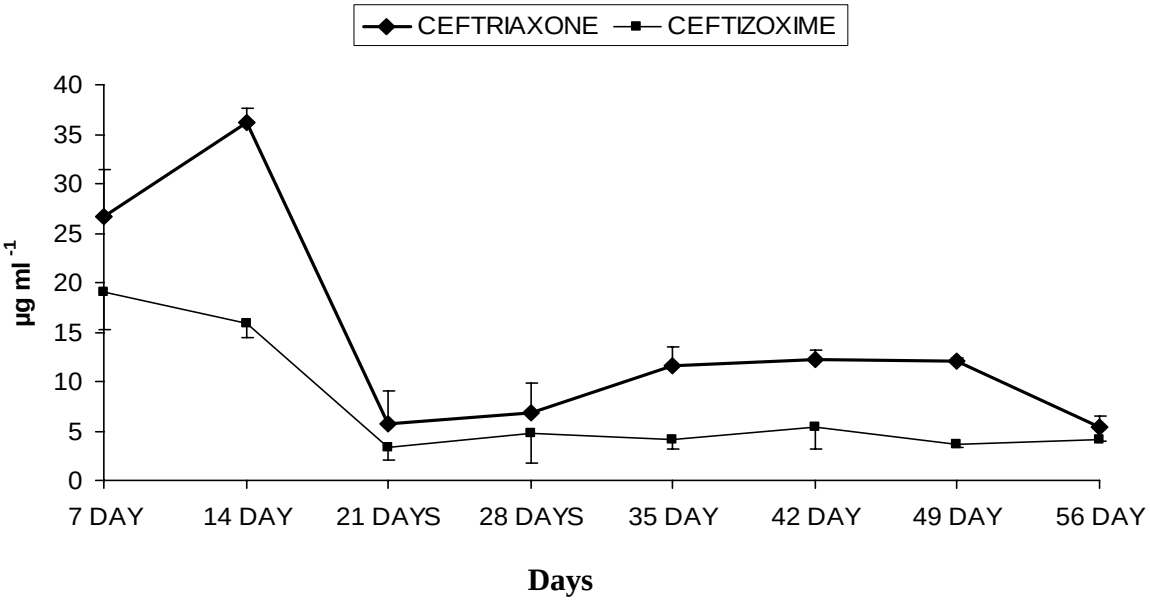


Fig. 6: Urine concentration of ceftriaxone and its metabolite (ceftizoxime) µg ml $^{-1}$ after consecutive daily intramuscular administration of ceftriaxone at 50 mg kg^{-1} for 60 days.

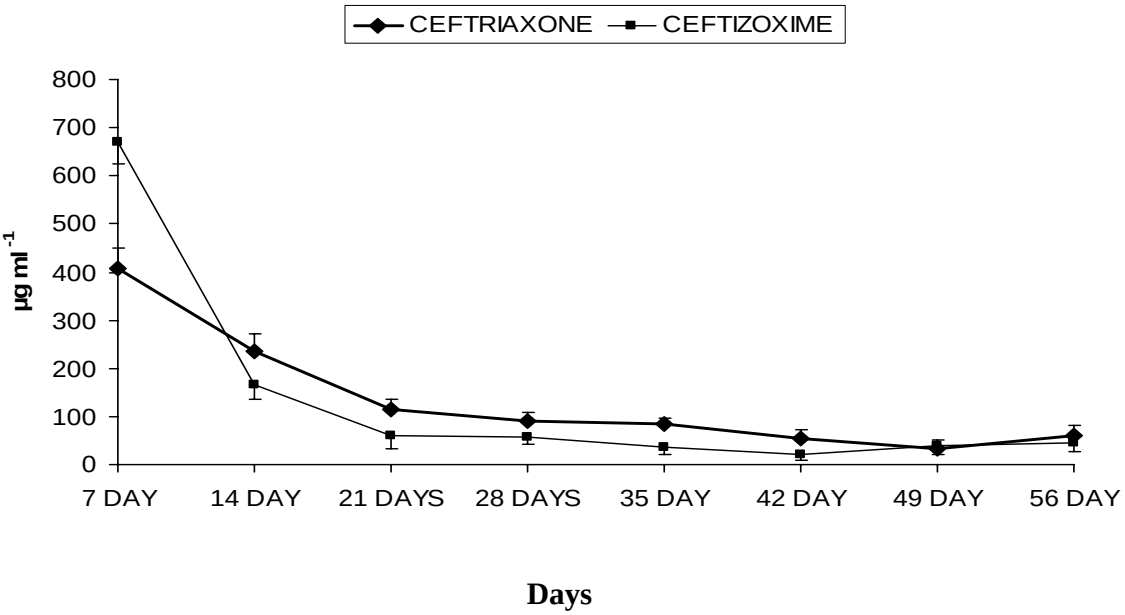
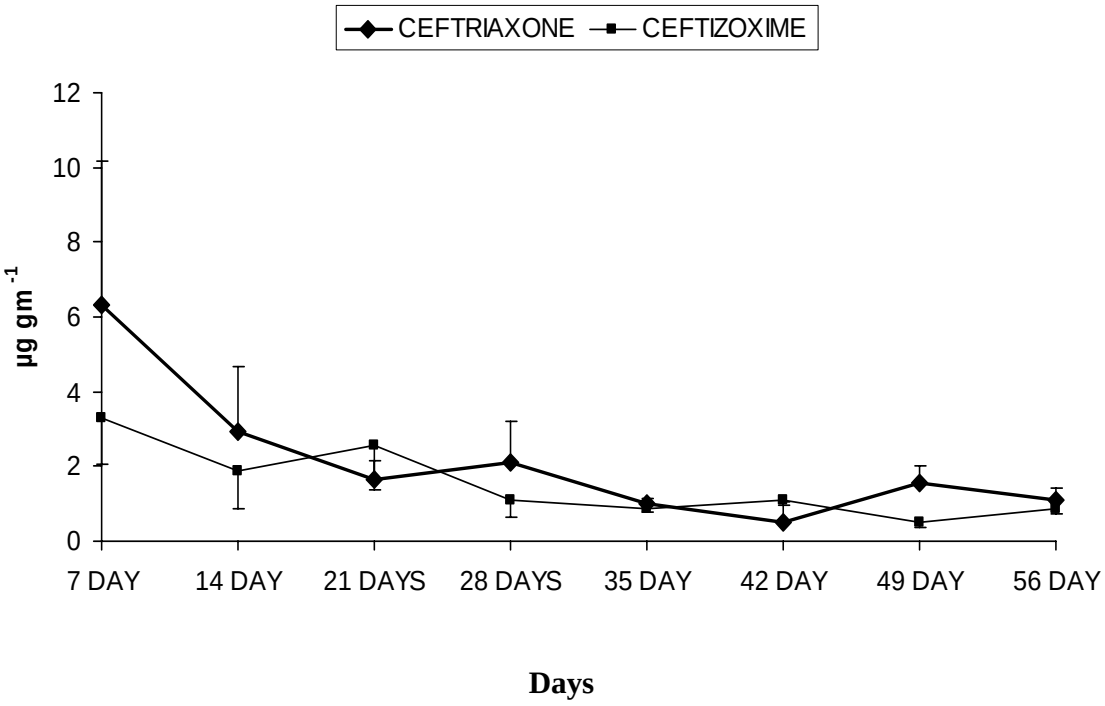


Fig. 7: Feces concentration of ceftriaxone and its metabolite (ceftizoxime) µg gm $^{-1}$ after consecutive daily intramuscular administration of ceftriaxone at 50 mg kg^{-1} for 60 days.



Tissues- Ceftriaxone and its metabolite (ceftizoxime) were widely distributed in all organs and tissues. Concentration of ceftriaxone, was high in kidney, lung and liver where as in brain, fat, bone, skin and muscle only ceftizoxime was identified. Animals sacrificed at day 67 after withdrawal of ceftriaxone administration for 7 days contain very 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 50 mg kg^{-1} dose level for 60 days.

	Sacrificed on day 60		Sacrificed on day 67	
Tissues	**Ceftriaxone**	**Ceftizoxime**	**Ceftriaxone**	**Ceftizoxime**
Kidney	11.87 ± 1.96	14.50 ± 1.47	0.50 ± 0.09	0.35 ± 0.03
Liver	2.28 ± 0.72	1.39 ± 0.32	0.81 ± 0.08	0.30 ± 0.05
Lung	8.98 ± 1.51	1.49 ± 0.09	0.79 ± 0.04	0.53 ± 0.08
Heart	0.60 ± 0.12	2.81 ± 0.18	0.19 ± 0.03	0.15 ± 0.02
Muscle	BDL	2.54 ± 0.22	BDL	0.16 ± 0.02
Skin	BDL	3.47 ± 0.26	BDL	0.11 ± 0.03
Bone	BDL	3.33 ± 0.26	BDL	0.22 ± 0.09
Spleen	1.58 ± 0.53	0.22 ± 0.02	BDL	0.11 ± 0.02
Fat	0.10 ± 0.03	3.56 ± 0.22	BDL	0.18 ± 0.02
Brain	BDL	5.77 ± 0.26	BDL	0.08 ± 0.01
Adrenal gland	0.83 ± 0.09	0.55 ± 0.08	0.09 ± 0.02	0.06 ± 0.01
Testis	0.77 ± 0.21	0.72 ± 0.11	0.24 ± 0.09	0.20 ± 0.06
Ovary	0.17 ± 0.04	3.21 ± 1.62	BDL	BDL
Uterus	BDL	0.58 ± 0.11	BDL	0.07 ± 0.01

Histopathology- Histopatholgoical examination of liver showed fatty changes in experimental animals but section of ovary, testis, kidney and lung of control and experimental groups did not reveal any histopathological changes. (Fig.8)

Biochemical parameters in blood- Biochemical studied showed significant increase ($p < 0.01$) of ALT activity in group II goats from day 35 onwards (Fig.9) and AST activity also increased in experimental groups from day 14 onwards (Fig 10). Plasma urea nitrogen values increased significantly ($p<0.01$) on day 21 onwards (fig.11) and plasma creatinine level also increased significantly ($p<0.01$) on day 42 onwards (Fig.12).

No significant differences were detected in blood glucose, serum protein and serum I_gG level at different days in experimental groups compared to control and respective '0' days (Fig. 13 to 15).

Tissue Biochemistry- No significant differences were also noticed in SOD, catalase, reduced glutathione and lipid peroxidation among goats of different groups (Table 2).

Fig. 8: Section of liver showing fatty changes in the hepatocytes of group II goats sacrificed on day 60 after consecutive daily intramuscular administration of ceftriaxone @ 50 mg kg-1 body weight.

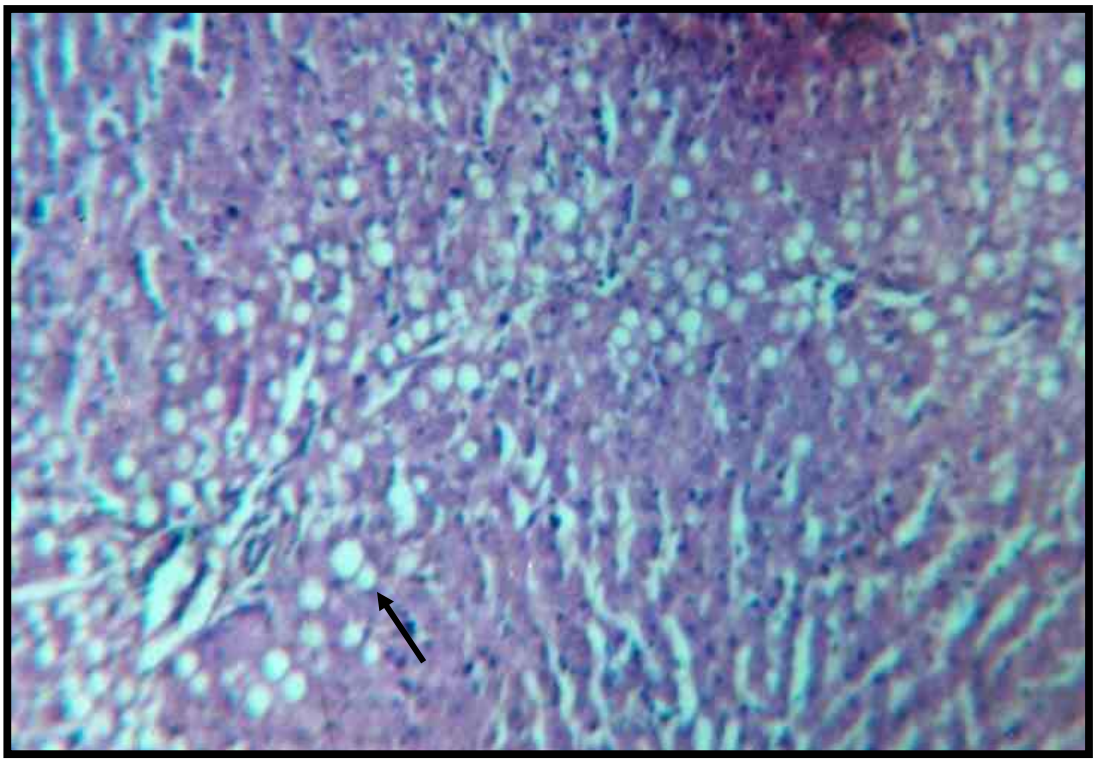


Fig : 9 Effect of ceftriaxone on serum alanine amino transferase (ALT) (µg pyruvic acid ml $^{-1}$ hr $^{-1}$) level in goats following consecutive once daily intramuscular administration at 50 mg kg $^{-1}$ body weight for 60 days

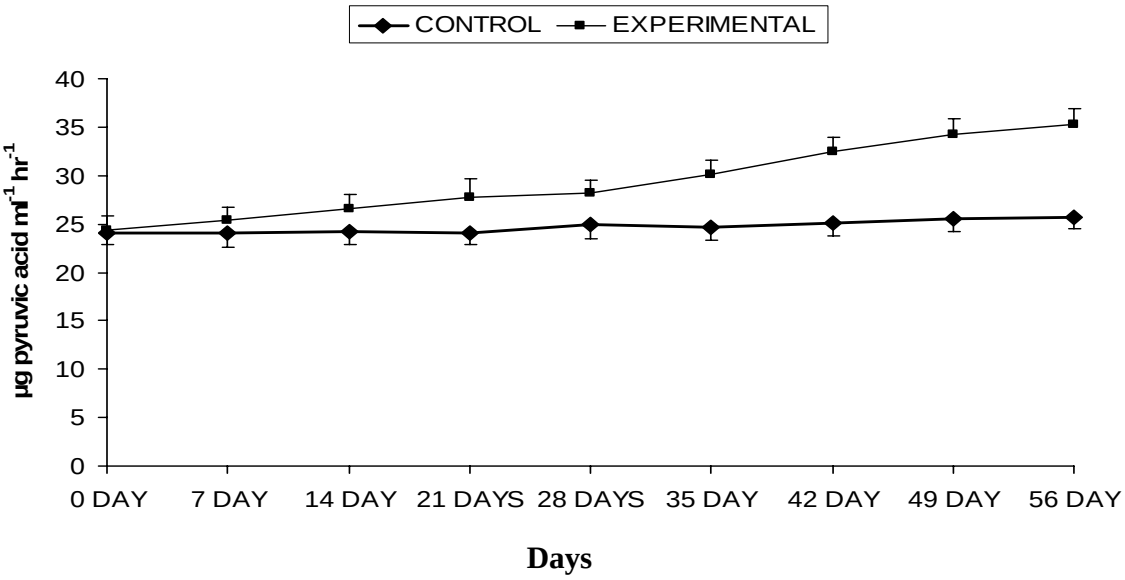


Fig. 10 Effect of ceftriaxone on serum aspertate amino transferase (AST) (µg pyruvic acid ml^{-1} hr $^{-1}$) level in goats following consecutive once daily intramuscular administration at 50 mg kg $^{-1}$ body weight for 60 days.

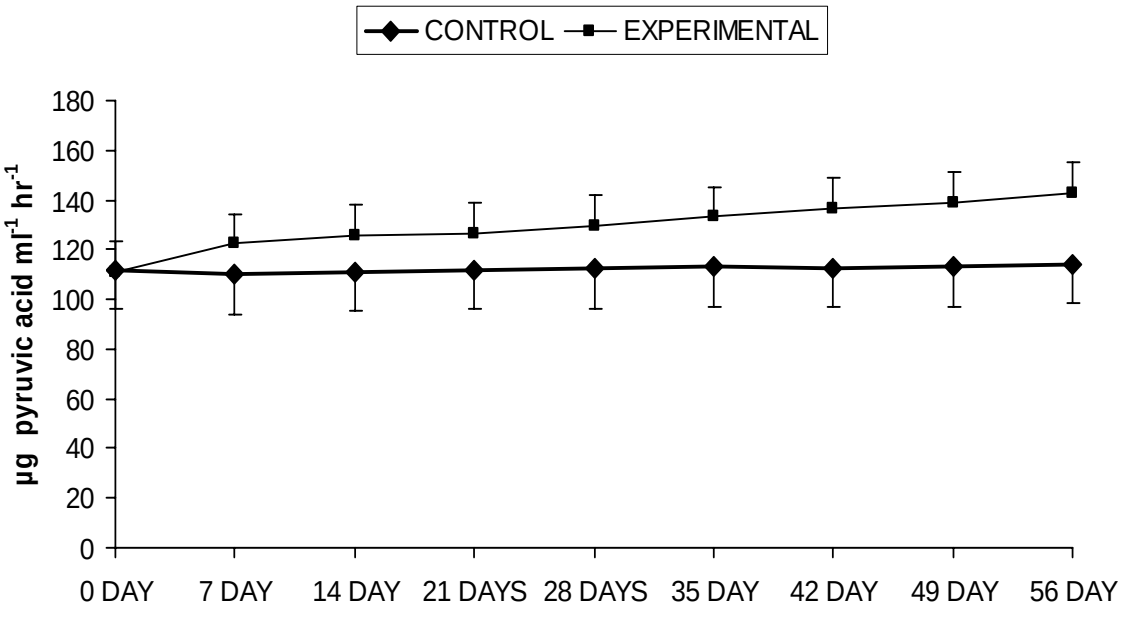


Fig. 11 Effect of ceftriaxone on plasma urea nitrogen (mg dl $^{-1}$) level in goats following consecutive once daily intramuscular administration at 50 mg kg $^{-1}$ body weight for 60 days

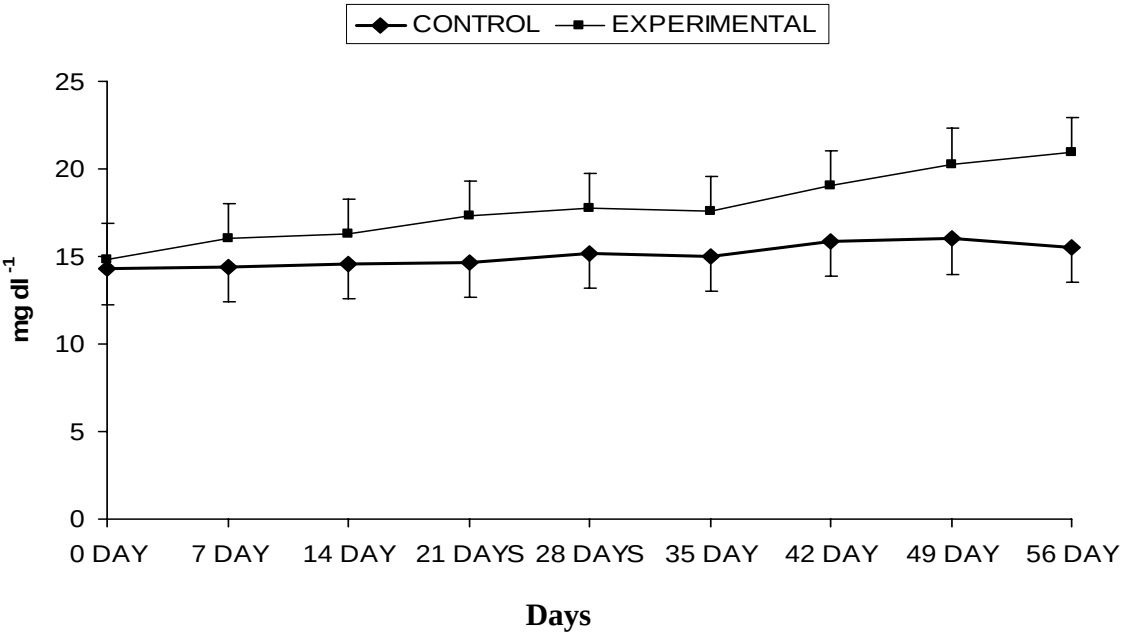


Fig. 12 Effect of ceftriaxone on plasma cretinine (mg dl $^{-1}$) level in goats following consecutive once daily intramuscular administration at 50 mg kg $^{-1}$ body weight for 60 days

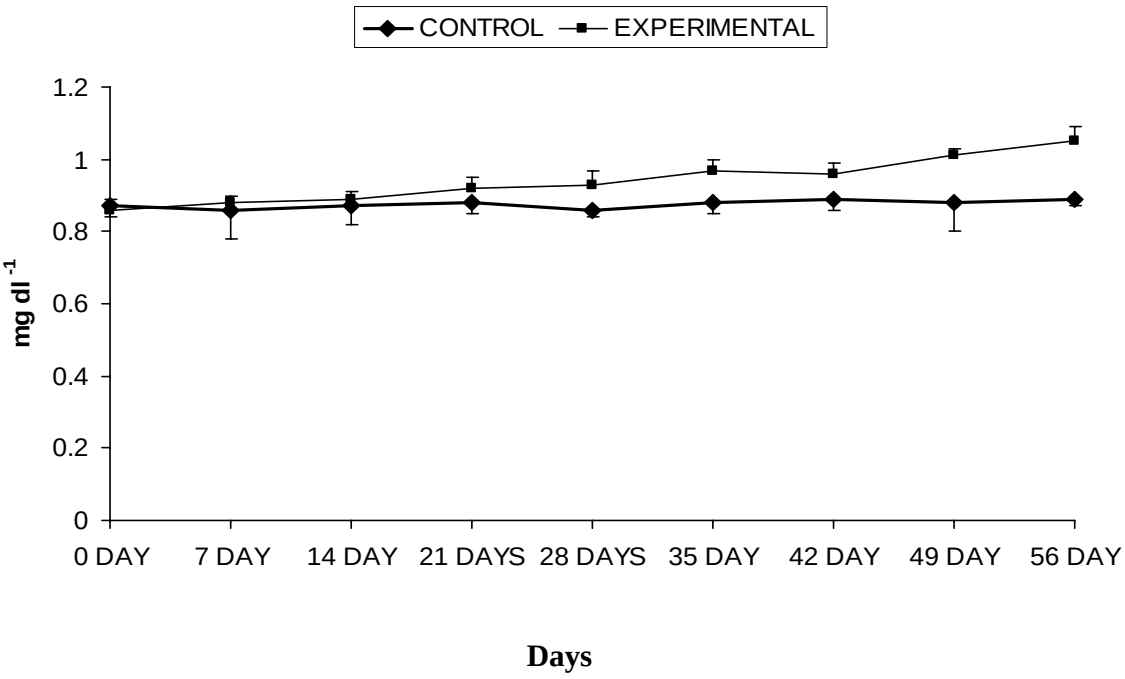


Fig.13 Effect of ceftriaxone on blood glucose (m mole L^{-1}) level in goats following consecutive once daily intramuscular administration at 50 mg kg^{-1} body weight for 60 days

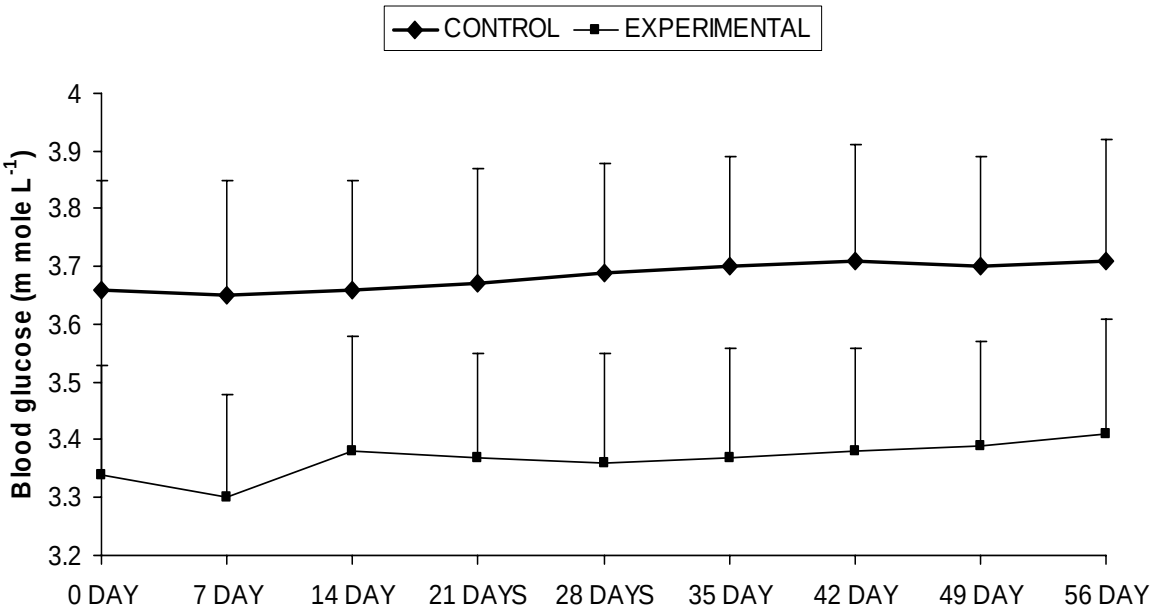


Fig. 14 Effect of ceftriaxone on serum protein (gm L^{-1}) level in goats following consecutive once daily intramuscular administration at 50 mg kg^{-1} body weight for 60 days

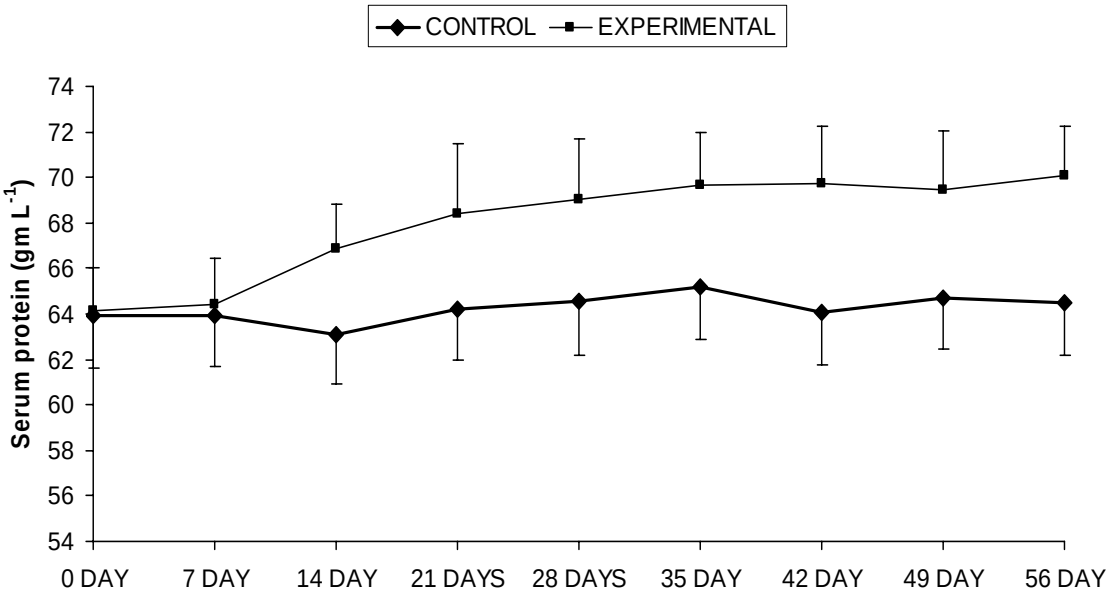


Fig. 15 Effect of ceftriaxone on serum immunoglobulin G (mg dl $^{-1}$) level in goats following consecutive once daily intramuscular administration at 50 mg kg $^{-1}$ body weight for 60 days

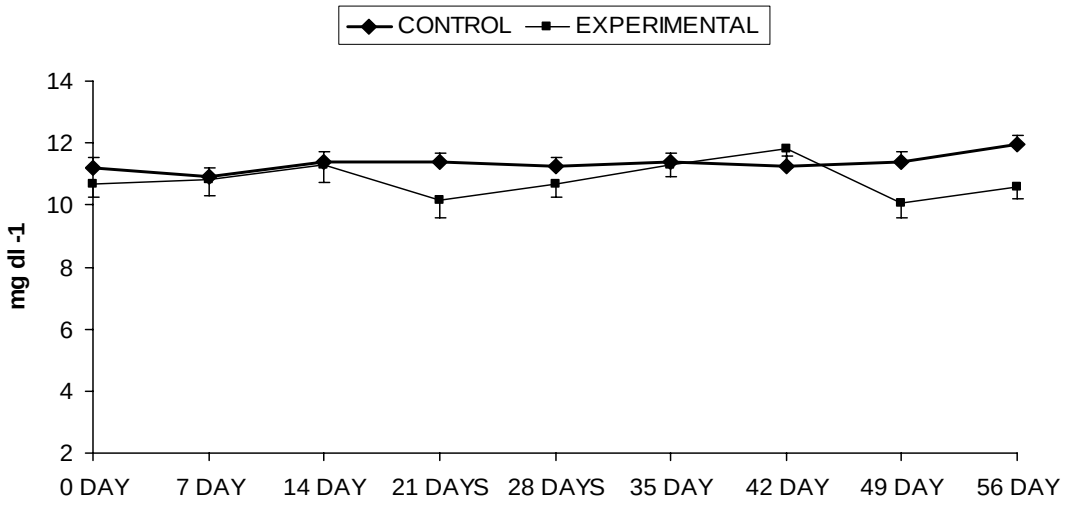


Table : 2 Effect of ceftriaxone on liver enzymes in goats following consecutive once daily intramuscular administration at two dose level for 60 days.

	Goats sacrificed on day 60		Goats sacrificed on day 67	
Parameters	**Control**	**Group II**	**Control**	**Group II**
Super Oxide Dismutase (Units mg $^{-1}$ of protein)	**0.68 ± 0.03**	**0.70 ± 0.04**	**0.64 ± 0.04**	**0.68 ± .04**
Catalase (mM H_2O_2 decomposed min $^{-1}$ mg $^{-1}$ of protein)	**0.34 ± 0.03**	**0.36 ± 0.02**	**0.35 ± 0.03**	**0.36± 0.04**
Lipid peroxidation (n mole of malonaldehyde gm $^{-1}$ of tissue)	**3.78 ± 0.16**	**3.98 ± 0.13**	**3.95 ± 0.19**	**4.00 ± 0.21**
Reduced glutathione (µ mole of glutathione gm $^{-1}$ of tissue)	**4.54 ± 0.39**	**4.64 ± 0.28**	**4.69 ± 0.42**	**4.71 ± 0.35**

Discussion

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 both ceftriaxone and ceftizoxime were decreased. Sar *et al.* (2006) [8] reported that ceftriaxone undergoes hydrolysis to form active metabolite ceftizoxime. Further, ceftriaxone, increased cytochrome P_{450} content in liver resulting in induction of microsomal mixed function oxidase system and therefore, microsomal hydrolysis was stimulated due to continued administration of ceftriaxone resulting in increase level of metabolite (ceftizoxime) and decreased level of ceftriaxone [8] But the concentration of ceftizoxime had also been decreased in the present study. Ceftizoxime is an active metabolite of ceftriaxone in goats [8] which may undergo biotransformation in liver to form inactive metabolite resulting in decrease concentration in blood. But the metabolism of ceftizoxime in goat has not been carried out in the present study. Therefore, further work on metabolism of ceftizoxime is warranted for its deceased level in blood of goats. A higher level of urea and creatinine is not a feature of primary tubular disease unless there is secondary glomerular diseases or ischaemia as an acute tubular necrosis [10]. No histopathological changes have been seen in section of kidney of ceftriaxone treated goats. Therefore it is difficult to discuss the reason of increased level of urea nitrogen and plasma creative level in the present study.

Permeability of hepatocyte may increase due to some pathological changes in liver tissues resulting escape of liver tissue enzyme like AST and ALT into circulating blood. Histopathological study showed fatty changes in liver which corroborated with increase AST and ALT level in serum.

Conclusion

Therefore it may be concluded from the above research work that prolong administration of ceftriaxone has no significant adverse effect in goats and consumption of chivon may not cause any public health hazard as the tissues devoid of residue after withdrawal period of 7 days.

Acknowledgement

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