International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 1018-1022
© 2018 IJCS
Received: 29-03-2018
Accepted: 30-04-2018

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Development of grade-II chronic renal failure model in black Bengal goat for pharmacokinetic studies

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Abstract
An animal model of chronic renal failure condition in caprine was created for pharmacokinetic studies. 15 goats were divided into 5 equal groups. Cisplatin (1mg ml^{-1}) administered at 30 mg m^{-2} intravenously bi-weekly for 28 days gave standard results for grade-II CRF. Blood Urea Nitrogen (BUN), Plasma Creatinine (CRT) and Glomerular Filtration Rate (GFR) were monitored for 91 days on weekly basis. BUN and CRT started to increase significantly from 7 days post dosing and maintained a significantly higher value till the end of the observation period. GFR decreased significantly on day 28 and maintained significantly lower value starting from day 28 to 91. The signs like emaciation, loss of body weight, and oliguria were observed. GFR was almost <60% up to 91 days. The signs like emaciation, loss of body weight, and oliguria were observed. The values of all 3 biomarkers and trucut biopsy confirmed Grade-II chronic renal failure in goats.

Keywords: Cisplatin, chronic renal failure, blood urea nitrogen (BUN), plasma creatinine (CRT), glomerular filtration rate (GFR)

Introduction
Renal impairment alters the normal disposition kinetics of some chemotherapeutic agents in human and animals. On the other hand, animals suffering from chronic renal failure at the same time may suffer from severe bacterial infection which needs treatment with broad spectrum antibiotics. So, pharmacokinetics of various antibiotics needs to be studied in chronic renal failure condition to pin point the changes in pharmacokinetic behavior of the drug in these diseased animals. But, there lacks adequate animal models for the renal failure in ruminants. Induction of acute renal failure in goats using uranyl nitrate has been reported Dutta *et al*. 2003) [7]. Cisplatin, an anticancer agent has been used in humans (Daugaard *et al*. 1990) [6]; for induction of acute renal failure in rats (Lee *et al*. 2004, Zhou *et al*. 2006) [10, 26] and chronic renal failure in goats (Mishra *et al*. 2013) [12]. The dose-limiting factor in cisplatin treatment is its well documented nephrotoxicity and neurotoxicity, the nephrotoxicity resulting in necrosis of proximal convoluted tubule (PCT) and collecting duct cells (Yao *et al*. 2007) [24]. Black Bengal goat, an indigenous breed of West Bengal and a prolific breeder which produces excellent quality meat and doesn't require much space and resources for its rearing. Therefore, goat is considered as the experimental animal which is well accepted as a prototype of cow for research purpose. Chronic renal failure is one of the major problems in goats. It is very difficult to find the efficacy or bioavailability of drug in experimental goats. Attempting to identify the primary process causing the kidney disease, especially in Stages I and II, is important to form a prognosis and treatment plan. Acute kidney disease may progress to a chronic condition; any cause of acute kidney disease is also a possible cause of chronic kidney disease (The Merck Manual, 2010) [21].

Materials and Methods
Experimental Animals
Clinically healthy fifteen Black Bengal female non-lactating adult goats (1-1/2 years age) weighing between 10-12 kg were used in this experiment. They were kept individually in the custom's made stainless steel metabolic cages (size 48'x48'x36'). The animals were stall fed and water was provided *ad libitum*.

The goat feed was supplied by EPIC, Kalyani, West Bengal. The temperature of the animal room was maintained at 25±2 ^{0}C and provided with artificial lighting facilities.

Before starting the experiment, the animals were de-wormed once with a mixture of Levamisol hydrochloride I.P. 1.5% w/v and Oxyclozanide I.P. (Vet) 3.0% w/v suspension (FLUZAN™, Jeps Pharma (p) Ltd.), C-207, Naraina Industrial Area, Phase -1, New Delhi- 110 028., at the total dose rate of 1ml/2kg b.w. After 21 days of de-worming, the animals were acclimatized in experimental environment for 7 days. The physical and clinical condition of the animals was observed throughout the experimental period. The study was approved by the Institutional Animal Ethics Committee, West Bengal University of Animal and Fishery Sciences, Kol-37.

Induction of Chronic Renal Failure Grade II

Chemical-induced kidney damage was carried out as per modifications to the method of Mishra *et al.* (2013) [12] for achieving Grade II Chronic Renal Failure where GFR is maintained below 60% of normal range for over a period of 3 months or longer. Fifteen goats were divided into 5 groups (I, II, III, IV and V) containing 3 animals each. Group I was kept as healthy control and animals of other groups received Cisplatin (1mg ml^{-1}) at different dosage levels. Group II received cisplatin at 30 mg m^{-2} intravenously once a week, Group III received cisplatin at 30 mg m^{-2} intravenously biweekly, Group IV received cisplatin at 30 mg m^{-2} intravenously thrice a week and Group IV received cisplatin at 30 mg m^{-2} intra venously four times a week for 28 days. Cisplatin (1mg ml^{-1}) administered at 30 mg m^{-2} intravenously bi-weekly for 28 days gave the standard results for grade-II Chronic Renal Failure.

Collection of Samples

Blood

Blood samples (2 ml) were collected in heparinised test tubes from jugular vein of animals and were centrifuged immediately at 3000 rpm for 20 min for separation of plasma which were further used for the estimation of plasma creatinine (CRT), blood urea nitrogen (BUN) and glomerular filtration rate (GFR).

Urine

Total excreted urine volume from each animal was measured and recorded throughout the experimental period. Urine samples (5ml) were collected and centrifuged at 2000 rpm for 20 min. Upper layer was collected and sediment was discarded. Collected urine samples were used for urine creatinine (CRT) and glomerular filtration rate (GFR) estimation.

Confirmation of Chronic Renal Failure

Induction of chronic renal failure after administration of cisplatin to animals was confirmed by the following biomarkers:

i. Blood Urea Nitrogen level (BUN)
ii. Plasma Creatinine level (CRT)
iii. Glomerular Filtration Rate (GFR)
iv. Ultrasound trucut biopsy of Kidney

Estimation of Blood Urea Nitrogen (BUN)

Blood Urea Nitrogen level was estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336, 338, 340, Road No.3, G.I.D.C., SACHIN 394 230 (Surat, India) as per DAM Method described by Coulambe *et al.* (1965) [5].

Estimation of Plasma creatinine level (CRT)

Plasma and Urine Creatinine level were estimated by using diagnostic kit (M/S Span Diagnostics Ltd. Plot No. 336,338,340,Road No.3,G.I.D.C., SACHIN 394 230 (Surat, India) as per Alkaline Picrate Method described by.

Determination of Glomerular Filtration Rate (GFR)

Glomerular Filtration Rate was determined by Creatinine Clearance (Cl_{crt}) method described by Cockcroft-Gault *et al.* (1976) [3].

Glomerular Filtration Rate (ml min^{-1} kg^{-1}) =

$$Cl_{crt} = \frac{U_{crt} \times V}{P_{crt} \times B.W.}$$

Where,

P_{crt} = Creatinine concentration in plasma

U_{crt} = Creatinine concentration in Urine

V = Urine excretion rate

Urine excretion rate was calculated for each animal of all groups by measuring the total excreted urine volume in metabolic cages over a period of 24 hrs.

Ultrasound trucut biopsy of Kidney

Histopathology of kidney tissue was performed by trucut biopsy technique. The sections from kidney were collected in one sacrificed animal post completion of experiment. Sample was processed in Hematoxylin and Eosin stain after processing the issue in microtome. TCB was performed using a Tru-Cut gun with an 18-gauge needle. After manual localization and immobilization of the lesion under complete aseptic technique, a 2% lignocaine-infiltrating anesthetic was administered and a skin incision was performed. A biopsy specimen was obtained by means of 4 successive insertions with different angulations of the needle into the lesion's core. After immediate immersion of the specimen in a fixative, its quantity and quality were judged and it was sent to the histopathology department.

Results and Discussion

Induction of chronic renal failure was done as per modifications to the method of Mishra *et al.* (2013) [12]. Standard results were obtained by intravenous administration of Cisplatin 1 mg ml^{-1} at dose rate of 30 mg m^{-2} bi-weekly for 28 days. Blood Urea Nitrogen, Plasma creatinine and Glomerular Filtration Rate were monitored for 91 days at 7 days interval following first intravenous dosing of cisplatin.

Blood Urea Nitrogen and Plasma creatinine values increased gradually from day 7 and maintained significantly higher value till the end of the observation period (day 91) (Table 1).On the other hand Glomerular Filtration Rate decreased significantly on day 28 and maintained significantly lower value starting from day 28 to day 91 (Table 1).

Table 1: Effect of cisplatin on Blood urea nitrogen (mg dl^{-1}), Plasma creatinine (mg dl^{-1}) level and Glomerular filtration Rate (ml min^{-1} kg^{-1}) in goats following Cisplatin administration at 30 mg m^{-2} intravenously bi-weekly for 28 days.

Days	Parameters		
	Blood Urea Nitrogen (mg dl^{-1})	Plasma creatinine (mg dl^{-1})	Glomerular Filtration Rate (GFR) (ml min^{-1} kg^{-1})
0	14.68 ± 0.23^{a}	0.74 ± 0.02^{a}	2.57 ± 0.03^{a}
7	16.54 ± 0.18^{b}	0.88 ± 0.05^{a}	2.45 ± 0.04^{a}
14	17.51 ± 0.12^{bc}	0.96 ± 0.04^{a}	2.36 ± 0.04^{a}
21	17.87 ± 0.06^{bc}	1.02 ± 0.04^{ab}	2.13 ± 0.07^{a}
28	18.40 ± 0.11^{cd}	1.16 ± 0.04^{bc}	1.92 ± 0.09^{b}
35	22.13 ± 0.17^{ef}	1.23 ± 0.04^{bc}	1.55 ± 0.20^{b}
42	23.28 ± 0.17^{f}	1.33 ± 0.03^{bc}	1.06 ± 0.12^{bc}
49	24.13 ± 0.10^{fg}	$1.45\pm0.05b^{c}$	0.91 ± 0.05^{c}
56	23.40 ± 0.15^{f}	1.57 ± 0.06^{bc}	0.89 ± 0.04^{c}
70	22.53 ± 0.14^{ef}	1.45 ± 0.07^{bc}	0.88 ± 0.06^{c}
84	21.96 ± 0.19^{e}	1.38 ± 0.07^{bc}	0.94 ± 0.07^{c}
91	21.08 ± 0.23^{e}	1.31 ± 0.06^{bc}	1.01 ± 0.07^{bc}

Values are Mean ± SE, n=3 in each groups.
Mean value with dissimilar superscript (^{a,b,c}) in the column vary significantly ($P<0.05$)

Biomarkers

Blood urea nitrogen

Mean values with SE of blood urea nitrogen level in goats following bi-weekly intravenous administration of cisplatin for 28 days at 30 mg m^{-2} have been depicted in Table 1 and Figure 1. Blood urea nitrogen level in goats before administration of cisplatin ('0' day) was 14.68±0.23 mg dl^{-1}, which started to increase from day 7 (16.54±0.18 mg dl^{-1}), peaked on day 49 (24.13±0.10 mg dl^{-1}) and maintained a plateau till 70th day. BUN level was significantly ($P<0.05$) higher in all the samples from day 7 onwards and up to day 91 post dosing of cisplatin compared to 0 day (Table 1).

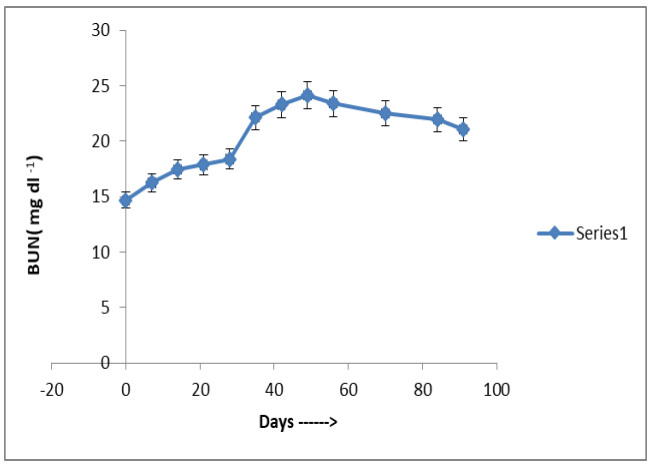

Fig 1: Effect of cisplatin on Blood Urea Nitrogen (mg dl^{-1}) level in goats following Cisplatin administration at 30 mg m^{-2} intravenously bi-weekly for 28 days.

Plasma creatinine

Mean values with SE of plasma creatinine level in goats following bi-weekly intravenous administration of cisplatin for 28 days at 30 mg m^{-2} have been presented in Table 1 and Figure 2. Plasma creatinine (CRT) level in goats before administration of cisplatin (0 day) was 0.74±0.02 mg dl^{-1}. The value was observed to increase from day 7 (0.88±0.05 mg dl^{-1}) and reached a maximum concentration of 1.57±0.06 mg dl^{-1} on day 56. The CRT level was significantly ($P<0.05$) higher in all the samples collected from 1 day onwards and up to day 91 post dosing of cisplatin compared to control sample of day 0 (Table 1).

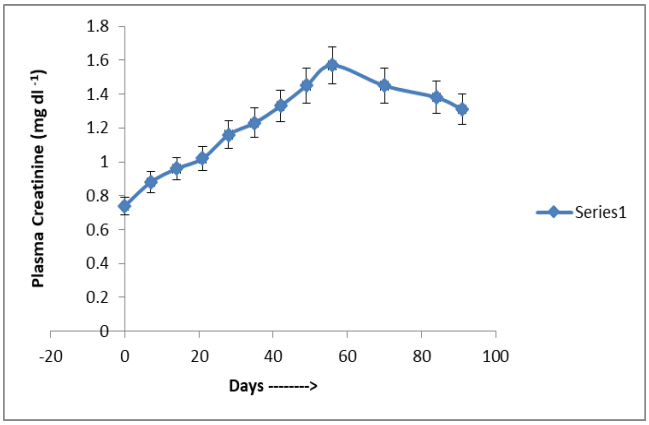

Fig 2: Effect of cisplatin on Plasma creatinine (mg dl^{-1}) level in goats following Cisplatin administration at 30 mg m^{-2} intravenously bi-weekly for 28 days.

Glomerular Filtration Rate

Mean values with SE of glomerular filtration rate in goats following bi-weekly intravenous administration of cisplatin for 28 days at 30 mg m^{-2} have been incorporated in Table 1 and Figure 3. Glomerular filtration rate (GFR) in goats before administration of cisplatin (0 day) was 2.57±0.03 ml min^{-1} kg^{-1}, which was gradually declined significantly ($P<0.05$) from day 7 (2.45±0.04 ml min^{-1} kg^{-1}) up to day 42 (1.06±0.12 ml min^{-1} kg^{-1}) of cisplatin treatment compared to control sample day 0.After that it maintained a steady plateau till 91 day (1.01±0.07 ml min^{-1} kg^{-1}) (Table 1).

The blood urea nitrogen (BUN) and plasma creatinine (CRT) are the commonly used biomarkers to study intensity of renal failure due to the fact that it is released in to the plasma at relatively constant rate in healthy state, which are freely filtered by the glomerulus and neither metabolized nor released by the kidney. The actual plasma creatinine may be increased or decreased, independent of changes in the glomerular filtration rate, by inhibiting or stimulating renal tubular secretion. glomerular filtration rate and renal gamma glutamyl transferase are the active biomarkers of chronic kidney diseases. Cisplatin appears to enter cells by diffusion. The chloride atoms may be displaced directly by reaction with nucleophiles such as thiols; replacement of chloride by water yields a positively charged molecule and is probably

responsible for formation of the activated species of the drug, which then reacts with nucleic acids and proteins, causing necrosis and damage to proximal tubule. Development of necrotic and fibrotic lesion at the corticomedullary junction is observed due to increased blood urea nitrogen and plasma creatinine levels. Wainford *et al*. studied the mechanism of cisplatin causing nephrotoxicity *in vivo* and *in vitro* model system. Nephrotoxicity was induced in rats (6 mg kg^{-1} cisplatin i.p.) and mice (10 mg kg^{-1} cisplatin i.p.). Cisplatin administration significantly elevated BUN and serum creatinine in male Sprague Dawley rats on day 5 post treatment and in male mice on day 4 post treatment where nephrotoxicity was confirmed by histological changes that showed significant damage to the proximal tubules of cisplatin versus saline vehicle treated animals. Chronic renal failure is developed due to irreversible chronic tubular necrosis by cisplatin injection without showing any symptoms of nephropathy in experimental goats. Arany *et al*. [1] showed that the major dose limiting side effect is nephrotoxicity, which evolves slowly and predictably after initial and repeated exposure. The kidney accumulates cisplatin to a higher degree than other organs perhaps via mediated transport. Functionally, reduced renal perfusion and a concentrating defect characterize its nephrotoxicity, whereas morphologically necrosis of the terminal portion of the proximal tubule and apoptosis predominantly in the distal nephron characterize its effects on cell fate. Daugaard *et al.* [6] studied the patho-physiological mechanisms involved in cisplatin induced nephrotoxicity. Immediately after administration of cisplatin to human, renal blood flow and re absorption rates decreased significantly. These data was confirmed in a micr- punture study in rats. After administration of 20 mg cisplatin per m^{-2} for 5 days in humans, a small but significant decrease in GFR was observed. In the high dose cisplatin group (40 mg m^{-2} for 5 days), a severe decrease in GFR was observed during treatment, and GFR remained decreased for up to 2 years after termination of treatment. The observation of an acute increase in N-acetyl-β-D-glucosamidase indicates a primary tubules effect of cisplatin in humans. These changes persist for at least 6 months after treatment. Therefore, a dose of 30 mg m^{-2} of cisplatin for bi-weekly intravenous administration in goats may be used for induction of chronic renal failure of model, which will persist till 90 days.

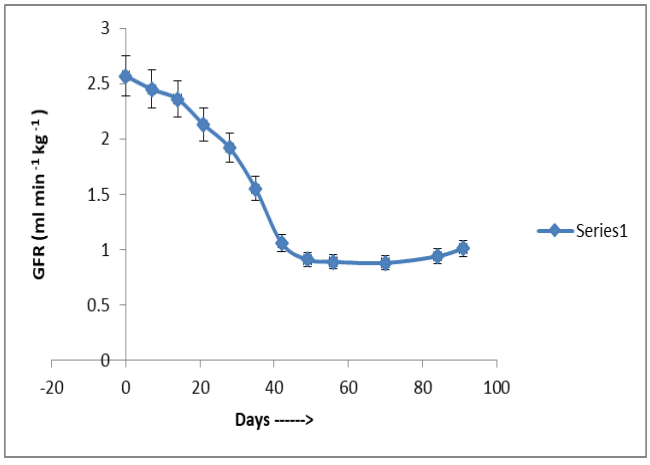

Fig 3: Effect of cisplatin on Glomerular Filtration Rate (GFR) (ml min^{-1} kg^{-1}) level in goats following Cisplatin administration at 30 mg m^{-2} intravenously bi-weekly for 28 days

Ultrasound trucut biopsy of Kidney

Section of kidney of test animal after administration of Cisplatin at 30 mg m^{-2} intravenously shows partial atrophic changes of glomeruli, degenerative changes in most of the tubules with necrotic changes Plate II compared to Plate I. On other hand, increased Bowman's space and no vascular damage were observed in Plate I. The proximal and distal convoluted tubules showed partial degeneration and loss of epithelia in Plate II whilst Plate I shows the section of kidney of animal of healthy normal architecture of parenchyma of kidney. Degenerative changes indicted an apparent trend of continued progression from reversible to the irreversible stage of cellular injury due to fluoride toxicity. Reversible injury was characterized by cellular swelling along with granular and vascular changes in lining epithelial cells of proximal convoluted tubules (PCTs) along with distal convoluted tubules (DCTs) and medullary tubules (MTs). Though the number of tubules so affected and a number of cells therein showing such changes varied greatly in the different microscopic area of section; more than 75% of PCTs and 50% of medullary tubules were consistently found to show such granular and vacuolar changes in at least more than 75% of their lining epithelial cells.

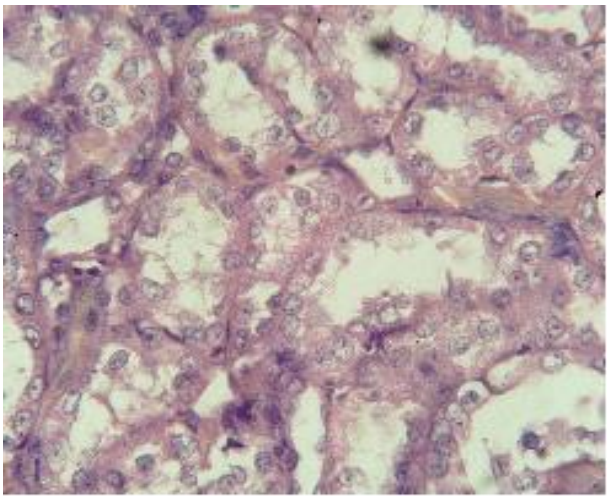

Plate I: A section of kidney showing healthy normal architecture of parenchyma of animal kidney (H and E, X40)

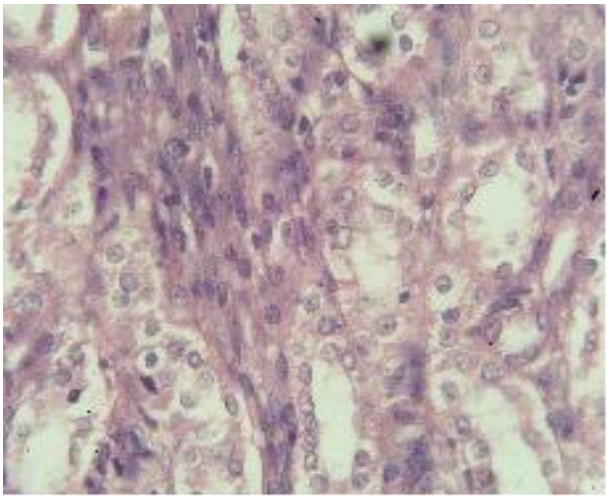

Plate II: A section of animal kidney showing partial atrophic changes of glomeruli, degenerative changes in most of the tubules with necrotic changes, PCTs and DCTs with partial degeneration and loss of epithelia (H and E, X40)

Acknowledgements
I sincerely acknowledge Dr. T. K. Sar, Associate Professor, Department of Veterinary Pharmacology & Toxicology, West Bengal University of Animal & Fishery Sciences and Dr. U.S. Chatterjee for his expert consultancy for the research work. Further I acknowledge the staff and seniors for their guidance during the research period. I would also like to acknowledge organizers of NRIPSCON-2017 for considering the work for oral abstract presentation held from 31st August to 2nd September, 2017 at KIET. School of Pharmacy (KSOP), Ghaziabad, Uttar Pradesh.

Funding Support
Dr. U.S. Chatterjee bestowed the Department of Pharmacology & Toxicology, West Bengal University of Animal & Fishery Sciences with the funding to procure the test drugs Cisplatin and Ceftizoxime for the study and the Department of Pharmacology & Toxicology for the purchase of animals.

References
1. Arany I, Safirstein RL. Cisplatin Nephrotoxicity. Seminars in Nephrology. 2003; 23(5):460-464.
2. Baggot JD. In: Mechanism of Drug Excretion, Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. Philadelphia: W.B. Saunders, 1977, 113-143.
3. Cockcroft DW, Gault MH. Prediction of Creatinine Clearance from Serum Creatinine. Nephron. 1976; 16(1):31-41
4. Cohen D, Gerald B, Appel BS, Neu HC. Pharmacokinetics of ceftriaxone in patients with renal failure and in those undergoing hemodialysis. Antimicrobial Agent and Chemotherapy. 1998; 24(4):529-532.
5. Coulambe GG, Favrean L. A. Clin. Chem., 1965; 11:624.
6. Daugaard G. Cisplatin nephrotoxicity: experimental and clinical studies. Danish Medical Bulletin. 1990; 37(1):1-12.
7. Dutta B, Mandal TK, Chakraborty AK. Pharmacokinetics of cefotaxime in goats with experimentally induced kidney damage. Indian Journal of Pharmacology. 2003; 35:173-176.
8. Goodman, Gilman. The Pharmacological Basis of Therapeutics, 10th Edn. Eds Hardman, J.G., Limbird, L.E. Molinoff, B.P., Ruddon, R.W. and Gilman, G.A. McGraw-Hill Co., New York, 2001.
9. Kowalsky SF, Echols RM, Vinezia AR, Andrews EA. Pharmacokinetics of ceftizoxime in subjects with various degrees of renal function. Antimicrobial Agents and Chemotherapy. 1983; 24(2):151-155.
10. Lee AS, Kang DG, Yaun JM, Woo WH, Kim YC, Sohn EJ *et al*. Butein ameliorates renal concentrating ability in disposition induced acute renal failure in rats. Biological Pharmacology Bulletin. 2004; 27(3):366-370.
11. Lee N, Yuen KY, Kumana CR. Clinical role of beta-lactam/beta-lactamase inhibitor combinations. Drugs. 2003; 63:1511-1524.
12. Mishra A, Chatterjee US, Mandal TK. Induction of Chronic Renal Failure in goats using Cisplatin: A new Animal Model. Toxicology International. 2013; 20:56-60.
13. Patel IH, Sugihara JG, Weinfeld EG, Wong C, OA Siemsen W, Steven Berman J. Ceftriaxone Pharmacokinetics in Patients with Various Degree of Renal Impairment. Antimicrobial Agent and Chemotherapy. 1984, 438-442.
14. Peterson LR, Gerding DN, VanEtta LL, Eckfeldt JH, Larson TA. Pharmacokinetics, Protein Binding and Extra vascular Distribution of Ceftizoxime in Normal subjects. Antimicrobial Agents and Chemotherapy. 1982; 22(5):878-881.
15. Polycarpe E, Arnould L, Schmitt E, Duvillard L, Ferrent E, Isambert N *et al*. Low urine osmolarity as a determinant of cisplatin-induced nephrotoxicity. International Journal of Cancer. 2004; 111(1):131-7.
16. Radiostits OM, Blood DC, Gay CC. Veterinary Medicine. A Text Book of The Disesases of Cattle, Sheep, Pigs, Goats and Horses. 10th Edn. Bailliere Tindall, 24-28 Oval Road, London. 1994, 442-7.
17. Saad YS, Tawfeeg AON, Ayman MN. Effects of gemcitabine on cisplatin-induced nephrotoxocity in rats: schedule-dependent study. Pharmacological Research. 2001; 43(2):193-198.
18. Sar TK, Mandal TK, Das SK, Chakraborty AK. Pharmacokinetics of ceftriaxone in carbon tetrachloride-induced hepatopathic and uranyl-nitrate induced nephropathic goats after single dose intravenous administration. Drug Metabolism Letters. 2008; 2:23-28.
19. Shaktidevan RK, Jha KC, Sar TK, Das SK, Chatterjee US, Chakraborty AK, Mondal TK. Effect of induced surgical stress and acute renal failure on disposition kinetics of ceftriaxone in goats. Indian Journal of Pharmacology. 2005; 37(3):186-188.
20. Snedecor GW, Cochran WG. Statistical Methodss. Iowa State University Press, Ames, IA, 1976.
21. The Merck Veterinary Manual. 51th Anniversary Edition; Published by Merck & Co., Inc White House Station, N.J., U.S.A, 2010.
22. Wainford RD, Weaver RJ, Stewart KN, Brown P, Hawksworth GM. Cisplatin nephrotoxicity is mediated by gamma glutamyltranspeptidase, not via a C-S lyase governed biotransformation pathway. Toxicology. 2008; 249:184-193.
23. Yamate J, Machida Y, Ide M, Kuwamura M, Kotani T, Sawamoto O *et al*. Cisplatin-induced renal interstitial fibrosis in neonatal rats, developing as solitary nephron unit lesions. Toxicologic Pathology. 2005; 33(2):207-17.
24. Yao XK, Panichpisal NK, Nugent K. Cisplatin Nephrotoxicity: A review. American Journal of Medical Science. 2007; 334:115-124.
25. Yatazidis H. Measurement of Transaminase in serum. Nature. 1960; 18:79-80
26. Zhou H, Kato A, Miyaji T, Yasuda H, Fujigaki Y, Yamamoto T, *et al*. Urinary marker for oxidative stress in kidney in cisplatin-induced acute renal failure in rats. Nephrological Dialysis and Transplant. 2006; 21:616-62.