International Journal of Veterinary Science

www.ijvets.com P-ISSN: 2304-3075 editor@ijvets.com

RESEARCH ARTICLE

Novel Model for Renal Failure and Anaemia Induced by 5/6 Nephrectomy in Wistar Rat

Shilpesh Devada*, Maulik Patel, Vinay Mishra, Ramchandra Ranvir, Rajesh Sundar and Mukul Jain

Departments of Pharmacology and Toxicology, Zydus Research Center, Sarkhej-Bavla N.H. No. 8A, Moraiya, Ahmedabad-382210 Gujarat, India

ARTICLE INFO

Received: October 20, 2012
Revised: November 22, 2012
Accepted: November 27, 2012

Key words:
5/6 Nephrectomy
Anemia
Biochemical parameters
Heamatological parameters
Renal failure

***Corresponding Author**
Shilpesh Devada
shilpeshdevda@yahoo.com

ABSTRACT

The present study describes modification in method of subtotal surgical renal ablation in rat and the advantageous effects of change in method in terms of biochemical and haematological parameters when compared to other established methods of nephrotoxicity. Male rats underwent 5/6 surgical nephrectomy or sham operations in two steps. The animals were divided into 3 groups viz. Group-I (Sham Control; n=6), Group-II (the whole right kidney ablation on day 1 and 2/3 left kidney ablation was done on day 7; n=10) and Group-III (the whole right kidney ablation and 2/3 leftt kidney ablation was done on same day; n=10). The haematological parameters like Haematocrit (HCT), Hemoglobin (Hb) and reticulocytes (%) and biochemical parameters like serum creatinine and urea were measured before surgery and on day 14, 21 and 28 after surgery There were significant increase in serum creatinine and urea levels in day 14, 21 and 28 post surgery suggestive of nephrotoxicity whereassignificantly decreased values of heamatocrit, haemoglobin and significantlyincreased value of reticulocytes suggest anaemia due to renal damage. Changes observed in the present study is comparable to the surgeries performed on the same day. There were more significant results in both surgery performed on the same day (Group –III) when compared to whole right kidney ablation and 2/3 left kidney ablation (Group –II) and Sham control (Group-I). There was only 20% mortality observed in group-III compared to 40 % in groupII. This model will be useful for the researcher tostudy nephotoxicity and anaemia, within short time, with less mortality, less animal suffering as per animal ethical point consideration and better values of biochemical and haematological parameters in comparison to the established models of 5/6 renal ablation.

Cite This Article as: Devada S, M Patel, V Mishra, R Ranvir, R Sundar and M Jain, 2012. Novel model for Renal failure and Anaemia induced by 5/6 Nephrectomy in wistar rat. Inter J Vet Sci, 1(2): 83-88. www.ijvets.com

INTRODUCTION

Among human disorders, renal failure remains a serious health problem, and several animal models of renal failure have been developed to investigate various aspects of this syndrome (Eschbah and Dennis, 1980; Gagnon and Gallimore, 1988 and Zhang *et al*., 1996). Renal failure is characterized by the retention of nitrogenous metabolites such as urea, creatinine and other guanidino compounds, uric acid, and hippuric acid, which could be related to the clinical syndrome associated with renal insufficiency. In addition, patients with chronic renal failure are frequently complicated by anemia. Renal anemia deteriorates the quality of life for patients with chronic renal failure (Ifudu *et al*., 1996). Anemia of renal failure results from a number of factors such as shortened red blood cell survival, decreased bone marrow activity, an inappropriately low level of erythropoietin (EPO) or by blunted erythropoietin secretion from the kidney Butthe etiology of the renal failure influences the degree of anemia (Eschbach *et al.*, 1987).

In experimental animal studies, Renal failure is the most often induced by subtotal nephrectomy (remnant kidney model). This model may be achieved by either ligation of renal vessels supplying the renal poles or surgical removal of both renal poles followed by contralateral nephrectomy (Chow *et al.* 2003). The technical difficulties are obvious in both approaches: the ligation method gives heterogeneous results due to anatomical variations of the renal artery among different

rats (Liu *et al.* 2003) and the surgical one presents the risk of haemorrhage and higher mortality due to surgical complications (Boudet *et al.* 1978). Another way to produce experimental renal failure is the administration of nephrotoxic agents. Antibiotic adriamycin (Okuda *et al.* 1986), cisplatin (Heidemann *et al.* 1990), uranyl nitrate (Fukuda and Kopple 1980), aristolochic acid (Debelle *et al.* 2004), adenine (Yokozawa *et al.* 1986) have been evaluated for inducing renal damage. The available subtotal (5/6) nephrectomy procedure includes surgical removal of one kidney followed by removal of 2/3 portion of remnant kidney after a one week (Fleck *et al.*, 2006). The aim of our work was to describe the methodology of surgical subtotal nephrectomy in rat on the same day, to characterize basic features of this model including the progressive changes in hematological, biochemical parameters, mortality and to compare the results of both surgical procedures. There were no reports of the subtotal nephrectomy on the same day instead of giving a one week period.

Hence, the purpose of the present study was to develop an experimental model of anemia with renal failure in rat by two different methods of 5/6 nephrectomy and compare both methods in terms of hematological & biochemical parameters as well as histopathological changes of the remnant kidney.

MATERIALS AND METHODS

Experimental animals

Healthy young male Wistar rats 5-7 weeks (150±15g) obtained from Animal Research Facility of Zydus Research Centre, Ahmedabad, and were housed in IVC (Individually Ventilated Cage) under standard laboratory conditions which included temperature (25 ± 3 °C), relative humidity (30 to 70%), photoperiod (light and dark cycle of 12h each) with food and water provided *ad libitum*. The protocol of the study was approved by Institutional Animal Ethics Committee (IAEC). All animals were acclimatised for five days prior to starting of experiments.

Experimental design

Groups	No of Animals (n)	Type of Surgery	Animal ID
I	6	Surgical control(Sham Operated)	1-6
II	10	Whole Rt Kidney removedon firstDay+ 2/3rd Lt Kidney removed after a week	7-16
III	10	Whole Rt Kidney + 2/3rd Lt Kidney removedon same day	17-26

Anaesthesia

Combination of 80 mg/kg ketamine and 10 mg/kg xylazine were given to anaesthetize the animals.

Surgical procedure for group II:

Phase I: The animal was anesthetized. The hair on the back of lumbar area was shaved. A cranial – caudal skin incision was made on the animal's right lateral to the spin with its cranial terminus just behind the rib cage. The kidney was freed from the surrounding tissue and was pull out of the incision gently. The adrenal gland which was attach loosely to the anterior pole of the kidney by connective tissue and fat, was gently made free by tearing the attachments, and was put back into the abdominal cavity. The renal blood vessels and the ureter were cauterized. The kidney was then removed by transecting the vessels and ureter just distal to the cauterized spot.

Phase II: One week after a first step, a ventral midline incision into the abdomen was made and sterile drape was applied. The intestine was retracted laterally to expose the animal's left kidney. The kidney was free from the surrounding tissue. A piece of suture was placed around each pole of the kidney at its 2/3 position. The sutures were gently ligated around the kidney. The 2/3 kidney on each end was excised right beyond the ligatures. The abdominal incision was closed with sutures and wound clips.

Surgical procedure for group III:

Same procedure was followed for group III, but the difference was that 5/6 portion of both the kidneys were removed on same day instead of giving a one week period between removal of whole right kidney and 2/3 portion of left kidney (Image 1).

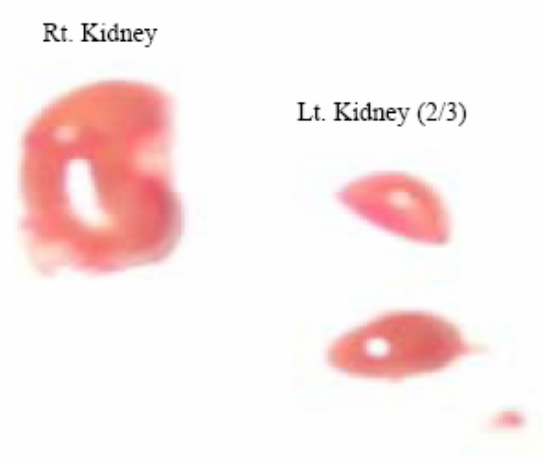

Image 1: Excised 5/6 Kidneys

Post operative care:

The cut surface of the remnant kidney was socked with the thrombin solution in order to prevent bleeding. Topical antiseptic ointment (Povidone iodine) and Ampoxin powder (Ampicillin) were applied on the sutured site to the animals which underwent surgery. Animals were observed for 28 days for any complications related to surgery.

Observations

(1) Animals were observed for clinical signs and mortality daily.

(2) Clinical Pathology: Detailed clinical pathological estimation on day 14, 21 and 28 after surgery were made under fasted conditions. Blood samples were collected from retro-orbital plexus.

Hematology

Whole blood was collected with 2% di-potassium EDTA, an anticoagulant and analyzed by using Cell-Dyn 3700 hematology analyser (Abott laboratories, USA).

Parameters evaluated were: Haematocrit (HCT), Hemoglobin (Hb) and reticulocytes %.

Serum Biochemistry

Biochemical analysis was done using Daytona autoanalyser (Randox Laboratories, UK). Details of parameters evaluated and the methods used are as follows: Creatinine (Alkaline picrate), Urea (Urease).

Histopathology

On day28 all the survived animals were humanely euthanized by carbon dioxide asphyxiation. Remnant kidney was collected and fixed in 10% formal saline, paraffin sections were prepared and stained with haematoxylin -eosin for histopathological examination.

Statistical analysis

All the values are presented as Mean ± SD. Student's-t test followed by unpaired t test was done between sham control and group II and III 5/6 Nephrectomized rats.

RESULTS

Clinical signs and Mortality:

At the end of study in sham control group all the animals survived whereas 2 animals on day 3 and another 2 animals on day 4 after surgery died in Group-II. 2 animals died in Group-III on day 2. Lethagy, decreased alertness and locomotor activity was observed in group II and III of 5/6 nephrectomized rats for the first seven days after surgery and then became normal. The feed and water consumption also decreased for first five days, but it was normal a week after surgery.

Clinical Pathology

Hematology

Haemoglobin levels was significantly decreased from 13.78 ± 0.41to12.78 ±0.59 & 12.08 ± 0.48 in group II nephrectomized rats at day 14 & 21 respectively after surgery when compared with sham control rats. A moresignificant reduction to 11.47 ± 0.98, 11.97 ± 0.52 & 12.24 ± 0.18 was seen in group III nephrectomized rats at day 14, 21 & 28 respectively after surgery (Table 1, Figure 1). HCT% was significantly decreased day dependently to40.00 ± 1.91 and 38.4 ± 1.87 in group II nephrectomizes rats at day 14 and 21 respectively in comparison with sham control rats which was towards normal at day 28 after surgery, whereas a reduction to the level of 36.53 ± 3.47, 38.02 ± 1.64 & 38.62 ± 0.75 was seen in group III nephrectomized rats at day 14, 21 and 28 respectively after surgery (Table 1, Figure 2). RTC% was significantly increased by 11.03 ± 3.99 and 7.27 ± 1.39 in group II nephrectomized rats at day 21 and 28 respectively in comparison with sham control rats, whereas a reduction to the level of12.85 ± 5.27, 11.68 ± 4.31 & 12.26 ± 3.85 was seen in group III nephrectomized rats at day 14, 21 and 28 respectively after surgery (Table 1, Figure 3).

Serum Biochemistry:

In nephrectomised rats urea was elevated to the levels of95.15 ± 31.12, 87.4 ± 26.12, 89.83 ± 31.19 and 96.63 ± 17.18, 85.6 ± 16.16, 79.92 ± 6.31 in group II and III at 14, 21 and 28 day respectively in comparison with sham control rats (Table 2, Figure 4).

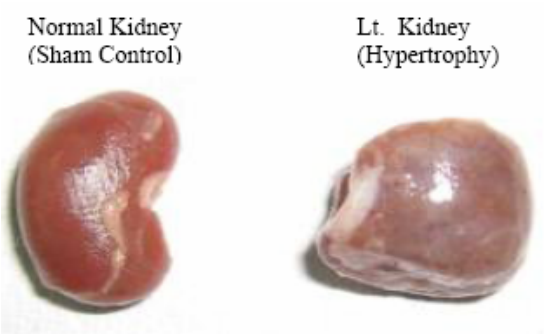

Image 2: Remnant hypertrophied left kidney at the end of 28 days post-surgery

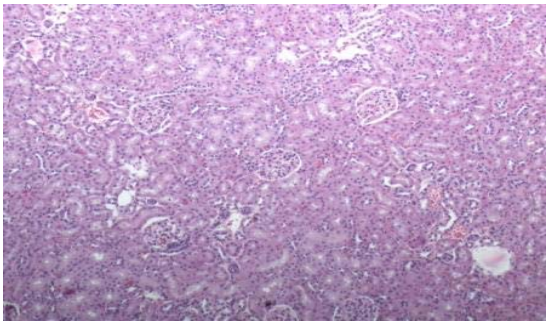

Image 3: Photograph showing normal glomeruli and renal tubules in sham control animals.

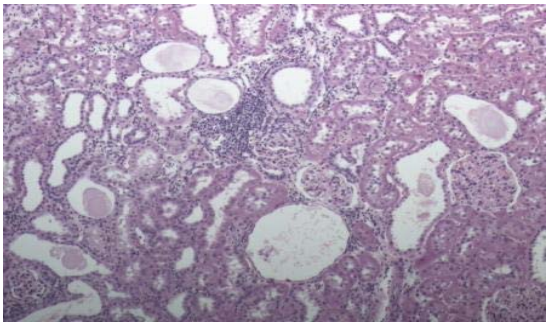

Image 4: Photograph showing mild tubular hypertrophy with mild interstitial fibrosis indicating moderate nephropathy in Group II.

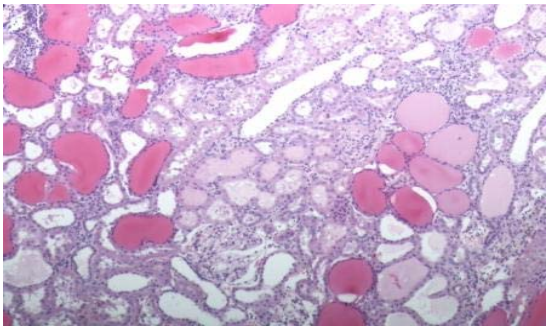

Image 5: Photograph showing mild tubular hypertrophy with mild interstitial fibrosis indicating moderate nephropathy in Group III.

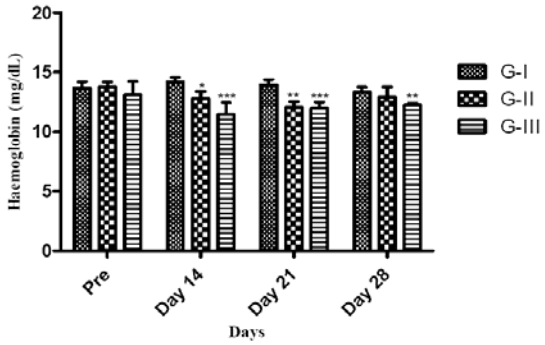

Fig. 1: Comparison of Haemoglobin levels (mg/dl) (mean±SD) before (pre) and on day 14, 21 and 28 after surgery within Group I, II and III respectively.

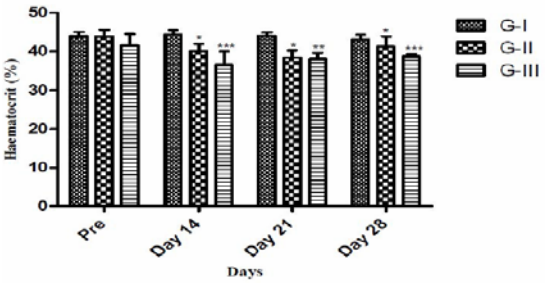

Fig. 2: Comparison of Haematocrit(%)values (mean ± SD) before (pre)and on day 14, 21 and 28 after surgery within Group I, II and III respectively.

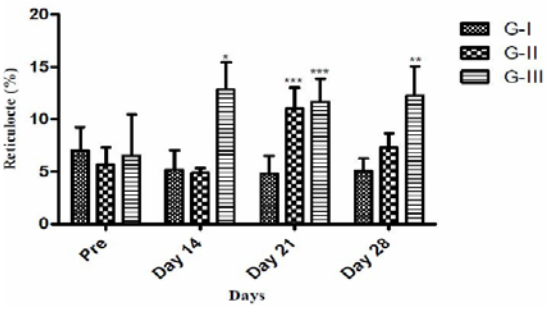

Fig. 3: Comparison of Reticulocyte(%) before (pre),and on day 14, 21 and 28 after surgery within groups I, II and III respectively.

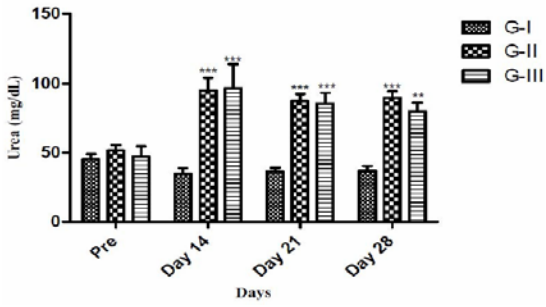

Fig. 4: Comparison of Urea (mg/dL) (mean ± SD) before (pre) and on day 14, 21 and 28 after surgery within groups I, II and III respectively.

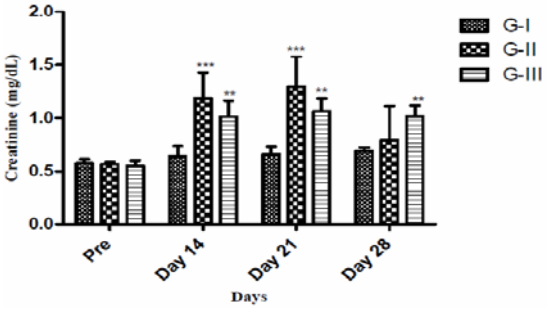

Fig. 5: Comparison of Urea (mg/dL) mean ± SD before surgery (pre) and on day 14, 21 and 28 after surgery within groupI, II and III respectively.

Creatinine level was significantly increased by 1.18 ± 0.24, and 1.29 ± 0.28 in group II at day 14 and 21 respectively in comparison with sham control rats in which it was towards normal at day 28 after surgery whereas an increased values 1.02 ± 0.15, 1.06 ± 0.12 and 1.02 ± 0.10 were seen in group III at day 14, 21 and 28 respectively after surgery (Table 2, Figure 5).

Gross and Histopathological examination of Kidney:

At the end of 28 days after surgery, there was increase in size of the remnant left kidney upon gross observation (Image 2) in survived rats. The remnant kidney was collected and subjected to histopathological examination which revealed, moderate nephropathy with mild interstitial fibrosis and mild tubular hypertrophy (Image 4 and 5) in group II and III when compared to normal glomeruli and tubules (Image 3) in sham control animals (group I).

DISCUSSION

In the present study, we used 5/6 part ablation of kidney on the same day to induce renal failure. Many other techniques have been reported to induce renal failure in animals. They include either surgical procedures such as ablation of part of the kidney tissue by 5/6 nephrectomy on different days in two different surgical phases (Fleck *et al.*, 2006) or ligation of the arteries (Vaneerdeweg *et al.*, 1992 and Levillain *et al.*, 1995), physical procedures using electrocoagulation (Gagnon and Gallimore, 1988) or cryosurgery, (Kumano *et al.*, 1986) or chemical procedures like administration of nephrotoxins via the bloodstream (Baehler *et al.*, 1977 and Nouwen *et al.*, 1994). Modifications in 5/6 renal ablation in rats by surgical removal of 5/6 kidney on the same day has not been reported in any literature. This nephrectomy model induces a decrease of functional renal mass entailing a uremic state. The haematological, biochemical parameters (Table 1 and 2) and gross and histopathological findings (Images 2, 4 and 5) illustrate the reproducibility of our model.

There were 40% and 20% mortality of animals observed in in Group II and Group III respectively which is comparable to the other surgical 5/6 renal ablation (Mumna *et al*, 1998). 20% less mortality in Group III gives superiority to this model. The death of animals occurred due to their inability to cope up the surgical

Table 1: Serum Biochemical parameters (mean ± SD).

Parameters	Groups	G-I	G-II	G-III
	No. of Animals	n=6	n=6	n=8
Urea (mg/dL)	Pre	45.43 ± 3.93	51.68 ± 4.00	47.4 ± 7.31
	Day 14	35.02 ± 3.90	95.15 ± 31.12 ***	96.63 ± 17.18 ***
	Day 21	36.45 ± 2.91	87.4 ± 26.12 ***	85.6 ± 16.16 ***
	Day 28	37.15 ± 3.39	89.83 ± 31.19***	79.92 ± 6.31**
Creatinine (mg/dL)	Pre	0.58 ± 0.04	0.57 ± 0.02	0.55 ± 0.05
	Day 14	0.65 ± 0.09	1.18 ± 0.24 ***	1.02 ± 0.15 **
	Day 21	0.66 ± 0.07	1.29 ± 0.28 ***	1.06 ± 0.12 **
	Day 28	0.69 ± 0.03	0.79 ± 0.32	1.02 ± 0.10**

*** indicates significant at p< 0.001 and ** indicates significant at p< 0.01.

Table 2: Haematological parameters (mean ± SD).

Parameters	Groups	G-I	G-II	G-III
	No. of Animals	n=6	n=6	n=8
Hb (mg/dL)	Pre	13.67 ± 0.53	13.78 ± 0.41	13.1 ± 1.12
	Day 14	14.2 ± 0.37	12.78 ±0.59 *	11.47 ± 0.98 ***
	Day 21	13.95 ± 0.45	12.08 ± 0.48 **	11.97 ± 0.52 ***
	Day 28	13.33 ± 0.45	12.95 ± 0.84	12.24 ± 0.18**
HCT%	Pre	43.92 ± 1.11	43.88 ± 1.70	41.47 ± 3.06
	Day 14	44.3 ± 1.15	40.00 ± 1.91*	36.53 ± 3.47***
	Day 21	43.97 ± 0.87	38.4 ± 1.87 *	38.02 ± 1.64 **
	Day 28	43.05 ±1.22	41.2 ± 2.69*	38.62 ± 0.75***
RCT%	Pre	7.01 ± 2.25	5.64 ± 1.65	6.53 ± 3.98
	Day 14	5.17 ± 1.89	4.91 ± 0.44	12.85 ± 5.27 *
	Day 21	4.80 ± 1.71	11.03 ± 3.99 ***	11.68 ± 4.31 ***
	Day 28	5.05 ±1.23	7.27 ± 1.39	12.26 ± 3.85**

***, ** & * indicates significant at p< 0.001, p, 0.01 & p, 0.1 respectively.

stress upon almost total kidney removal, which subsequently leads to excretory failure expressed in terms of generalised oedema and death. The survived animals showed weakness for first five days due to stress of kidney removal and surgery then become normal (Fleck *et al.*, 2006).

The values of haemoglobin and haematocrit were significantly lower in group III ($p<0.001$), II ($p<0.01$) than compared to Group I. The highly significant reduction in haemoglobin and haematocrit values of group III animals suggests the advantage of renal ablation on same day model than that of different day surgery model. These data are also comparable to the other well established methods of inducing nephrotoxicity (Yokozawa *et al.* 1986). Reticulocyte % increases more significantly with higher values and it persists longer for 28 days post-surgery in group III animals compared to group II animals, wherein reticulocyte % increases less significantly with lower values. The changes in haematological parameters in both groups II and III are indicative of the damage to the kidney as a result of which less erythropoietin production occurs which leads to reduction in haemoglobin, haematocrit and total erythrocyte count with arise in the reticulocytes (Dara *et al.*, 2009).

Similar results were observed in values of serum creatinine and urea, where more significant increase in serum creatinine levels was observed group III animals compared to group II animals. These higher values persisted for 28 days post-surgery in group III, whereas it remained higher up to day 21st and subsequently decreases towards normal on day 28th of post-surgery in group II. The level of urea in both group II and III were increased highly significantly and this higher levels persisted up to the end of study. These increased values of serum creatinine and urea have shown similarity to that of other models for inducing nephrotoxicity. (Chow *et al.*, 2003). Rise in levels of serum creatinine and urea detected in 5/6 renal ablation, is due to more than 75% kidney damage induced.

The size of the remnant right kidney was found to be increased due to the compensatory hypertrophy in both group II and III. Histopathological examination of the remnant right kidney revealed moderate nephropathy with mild interstitial fibrosis and mild tubular hypertrophy in both group II and III, which is comparable with the other methods of nephrotoxicity(Marina *et al.*, 2003).

Conclusion

The result of our study showed that the renal failure induced by 5/6 nephrectomy on same day might be useful to producemore significant results in terms of haematology, biochemistry and gross tissue changes. This modification in renal ablation on same day will save the time and gives good or equivalent quality results in comparison with other methods of inducing renal toxicity. The model might be an interesting tool for further pathophysiological and behavioural investigations particularly with regard to the excretory failure, uremic syndrome and induction of anaemia.

Acknowledgements

Authors sincerely acknowledge each and every team member of Toxicological Section of Zydus Research Centre for their valuable support in completing this work.

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