Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 4 Issue 4 April 2022

Research Article

Use of Hemostatic Compression in the Making of the Nx 5/6 Experimental Model in Wistar Rats

Fellipe Ferreira Lemos de Medeiros[1], Natasha Nogueira Ferreira[2], Cecília Ribeiro Castañon[2], Patricia Pereira Almeida[3], Ágatha Cristie Menezes[3], D'Angelo Carlo Magliano[4], Milena Barcza Stockler-Pinto[4], Maria de Lourdes Gonçalves Ferreira[5], Viviane Alexandre Nunes Degani[6]* and Nayro Xavier de Alencar[5]

[1]*Postgraduate Program in Clinic and Animal Breeding, Universidade Federal Fluminense, Niterói, RJ, Brazil*

[2]*Autonomus Veterinarian, Niterói-RJ, Brazil*

[3]*Postgraduate Cardiovascular Sciences Post Graduate Program, Fluminense Federal University (UFF), Niterói-RJ, Brazil*

[4]*Cardiovascular Sciences Post Graduate Program, Fluminense Federal University (UFF), Niterói-RJ, Brazil*

[5]*Clinic and Animal Reproduction Post Graduate Program, Fluminense Federal University (UFF), Niterói-RJ, Brazil*

[6]*Morphology Department, Biomedical Institute, Fluminense Federal University (UFF), Niterói-RJ, Brazil*

***Corresponding Author:** Viviane Alexandre Nunes Degani, Morphology Department, Biomedical Institute, Fluminense Federal University (UFF), Niterói-RJ, Brazil.

DOI: 10.31080/ASVS.2022.04.0359

Received: March 01, 2022

Published: March 22, 2022

Abstract

Objectives: To enhance the technique for the creation of the experimental model for chronic kidney disease with 5/6 nephrectomy and to establish parameters for the postoperative evaluation of animals undergoing the surgical procedure.

Methods: Twenty-one male Wistar rats aged over two months were divided into two groups, namely, SHAM (n = 9) and 5/6 Nx (n = 12). The animals were anesthetized intraperitoneally with ketamine, midazolam and tramadol. In the 5/6 Nx group, bipolar nephrectomy of one kidney and total nephrectomy of the contralateral kidney were performed in a single surgical procedure, whereas the SHAM group underwent laparotomy without nephrectomy. The following measurements were carried out in both groups: 24-h urinary volume and proteinuria; total body weight; systolic blood pressure; and serum urea and creatinine.

Conclusion: The modifications in the technique proved to be feasible, providing longer survival of the animals in the medium term, fewer intraoperative complications and effectiveness for the construction of the experimental model.

Keywords: Nephrectomy; Kidney Disease; Rodents

Introduction

Chronic kidney disease (CKD) encompasses several pathological processes associated with abnormal kidney function and a progressive decline in glomerular filtration rate [1,2]. With a global prevalence of 13%, CKD is considered a global public health problem. Its growth is evidenced by the increase in deaths attributed to it as well as by the incidence and prevalence of the end stage of the disease in the population [3,4]. The increasing occurrence of CKD in many countries is due to the older age of the human population and changes in lifestyle, along with the growing prevalence of obesity [5]. On the other hand, despite the high mortality caused by CKD and its greater presence in the population, clinical diagnosis and therapeutic interventions do not accompany this evolution [6].

Rats and mice have been extensively used to elucidate the pathogenesis and mechanisms of CKD [7].

In addition to being commonly used in the study of nephropathies, these experimental models with rodents are needed for the investigation of potential forms of diagnosis and therapeutic approaches, as well as for identifying biomarkers [6].

Nephrectomy was first performed in animals in 1889, by Tuffier, in canines. In the procedure, one kidney and part of the other kidney were removed with no changes in the elimination of water or urea [8]. In the same year, also with dogs, Bradford removed 3/4 of the total renal mass and reported death after one to six weeks, with coma and seizures [9].

MacNider [10] used uranium as a toxic therapy to cause nephritis in dogs and conduct an experimental study of renal failure. Three decades later, Heymann., *et al*. [11] caused renal injury in Sprague Dawley rats through immunologically induced nephritis. A similar method was used by Allison., *et al*. [12] in Wistar rats.

Immunological and toxic methods have been replaced by surgical methods, as the former can rarely be regarded as stable approaches for creating an experimental model of renal failure [13]. The remnant kidney model is one of the most commonly used for CKD. The procedure, which consists of total right nephrectomy and resection of the left renal poles, is called 5/6 Nx [6,14]. The method is based on the stages of CKD, which are defined based on the glomerular filtration rate. In stage 4 of the disease, there is a marked reduction in this rate, with only 15-29% being functional. Finally, in renal failure, or stage 5, less than 15% is functional [1].

Chanutin and Feris [15] were the first to remove 5/6 of the renal mass of rats, causing uremia that lasted for a long period. The pioneering surgery consisted of two surgical phases: firstly, ligation of the renal poles, keeping them *in situ* in some cases and in others performing the resection, leaving one-third of the renal parenchyma and the renal hilum intact. After one to two weeks, contralateral nephrectomy was undertaken.

Patt., *et al*. [16] later performed the same procedure without ligation of the poles, only resection, followed by nephrectomy of the right kidney 10 to 14 days later. More recently, He., *et al*. [14] also performed the same procedure on the left kidney, allowing it to recover for a week for subsequent removal of the right kidney after ligation of the renal artery and vein and the ureter.

In Sprague Dawley rats, in addition to unilateral right nephrectomy, Avioli., *et al*. [17] ligated most of the primary and secondary divisions of the left renal artery, causing renal infarction. This method was also used by Ergur., *et al*. [18], who caused partial infarction of approximately two-thirds of the left kidney by selective ligation of two of the three or four extrarenal branches of the renal artery. Hamzaoui., *et al*. [19] also based their experiment on the 5/6 Nx model.

Other means to establish the kidney disease model have been used. Boudet., *et al*. [20] used the electrocautery method to produce kidney injury, followed by contralateral nephrectomy in the second surgical phase. Kumano., *et al*. [13] induced kidney failure in Wistar rats by freezing the left kidney with liquid nitrogen at different times of exposure, causing different degrees of kidney damage, and performed right nephrectomy in a later procedure. A contributing factor to the widespread use of the method of eliminating 5/6 of the renal mass in rats is the persistent decrease in glomerular filtration rate, proteinuria, glomerular sclerosis and hypertension induction [21].

Aim of the study

Therefore, the aim of this study was adapted the technique described in the literature, searching for prove the efficiency of the technique, reducing animal mortality and prolonging the life of the experimental model, in addition to having fewer intraoperative complications.

Material and Methods

This experiment was approved by the Comitte on Ethics in the Use of Animals of Universidade Federal Fluminense with the number 956/18 in accordance with the welfare standards of animals use. Twenty-one male Wistar rats weighing between 400 and 450 g and aged over two months, from the Laboratory Animal Center (NAL/UFF), were selected. The animals were kept in the vivarium of the Experimental Nutrition laboratory at Universidade Federal Fluminense, under controlled temperature and light conditions, in individual nurseries with wood shavings where they received drinking water and feed *ad libitum*.

The rats were divided into two groups. The 5/6 Nx group underwent 5/6 nephrectomy (n = 12), whereas the control group

(SHAM) was subjected to the same anesthetic protocol and a similar process of dieresis and synthesis except for the reduction of renal mass but respecting the same surgical time as in the 5/6 Nx group (n = 9).

After weighing the animals, anesthetic induction was achieved with the association of ketamine (75 mg/kg), midazolam (10 mg/kg) and tramadol (4 mg/kg) intraperitoneally. After obtaining the anesthetic plan, trichotomy and antisepsis of the abdominal region were performed, with the animals in the supine position. Subsequently, surgical drapes were placed and fixed with Backhaus towel clamps. All surgical procedures were undertaken by the same surgeon, aiming at greater uniformity.

The animals were subjected to laparotomy through a median sagittal incision of approximately three centimeters in length, performed with a no. 11 carbon steel scalpel blade attached to a 13-cm no. 3 scalpel handle. Then, the subcutaneous tissue was carefully pulled apart using Adson forceps with 12-cm rat teeth and 14-cm straight Metzenbaum scissors, and the linea alba of the muscle layer was identified. Next, the buttonhole incision was performed with a scalpel and extended with 14-cm straight Metzenbaum scissors, giving access to the abdominal cavity.

After laparotomy, two 12-cm Allis forceps were used on each muscle edge of the incision and mild traction was applied to inspect the abdominal cavity, aiming to macroscopically evaluate the organs and verify the absence of alterations. After identifying the left kidney in the left retroperitoneal region and applying digital traction to it for better exposure, an incision was made in the retroperitoneal face with 14-cm straight Metzenbaum scissors and the vascular structures (renal artery and vein) and the ureter were identified. Ligation was performed in bloc near the renal hilum region with absorbable 4.0 polyglactin suture, followed by dieresis of the connected structures, ending total left nephrectomy.

In the right retroperitoneal region, the right kidney was identified, digitally exposed and incised in the outermost layer of the peritoneum that covers it, using 14-cm straight Metzembaum scissors. For the surgeon's visual assessment, the renal poles were identified, and the right kidney was divided into three parts of similar weights. A partial nephrectomy of the cranial pole was carried out by crushing it with straight 14-cm Kelly hemostatic forceps, aiming to reduce the bleeding caused by resection. After crushing, the cranial renal pole fragment was completely excised and the remaining parenchyma in the renal mass underwent hemostasis using a Hemospon® hemostatic sponge. Then, the procedure was repeated in the caudal renal pole. The fragments removed from the right kidney were evaluated by weight and compared with the total weight of the left kidney to confirm the removal of two-thirds of the right renal mass (Figure 1).

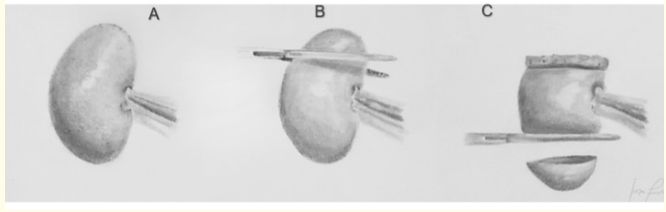

Figure 1: Schematic of partial nephrectomy procedure of (A) Wistar rat right kidney. (B) Positioning of the Kelly clamp on the cranial pole of the right kidney. (C) presence of the Hemospon hemostatic sponge on the cranial pole of the right kidney and crushing of the caudal pole with the presence of the removed fragment, UFF, 2019. From: Fellipe Ferreira Lemos de Medeiros.

After correct removal was verified, the remaining renal third was omentalized and repositioned in the abdominal cavity in the right retroperitoneal region. The synthesis of the incised layers of the abdomen was achieved with a simple running pattern absorbable suture of 4.0 polyglactin in the muscle; subcutaneously with a 14-cm Mayo-Hegar needle holder and 12-cm Adson forceps; and in the skin with non-absorbable nylon 4.0 suture, X stitch. The skin stitches were made without much tension, to avoid the animals' discomfort and prevent them from subsequently tearing the suture by them, which could cause dehiscence. After cleaning the surgical incision area with 0.9% NaCl saline, 1.0% chlorhexidine gluconate antiseptic was applied in spray form.

The rats in the SHAM group underwent the same laparotomy procedure, with the exposure of both kidneys with the same intraoperative duration as the animals in the 5/6 Nx group. Because nephrectomy was not performed, the kidneys were repositioned in the abdominal cavity and the same synthesis procedures were performed in both groups.

The animals were sent for immediate postoperative follow-up, in which they received analgesia with dipyrone 150 mg/kg subcutaneously for three days, tramadol hydrochloride 4 mg/kg subcutaneously for three days and antibiotic therapy with enrofloxacin 11 mg/kg subcutaneously in a single dose. Afterwards, they were laid in wood shavings beds during anesthetic recovery and later taken to their respective cages. Due to the great loss of renal mass, the animals did not receive anti-inflammatory medication.

After the surgical procedure, the animals of both groups were clinically evaluated for possible clinical signs such as hematuria, epistaxis, apathy and death. The animals which did not exhibit anesthetic recovery or died within the first 24 h had their death linked to a great loss of renal mass.

Body mass (in kilograms) was measured weekly, for 12 weeks, by weighing the animals on a scale. Systolic blood pressure was recorded with an Insight EFF 306 tail plethysmograph for rats and mice, in both groups, at the fifth, sixth, eighth, tenth and twelfth postoperative weeks. The rats were placed in metabolic cages for 24-h urine collection for urinary volume and proteinuria analysis at the fifth and twelfth weks after surgery. A venous blood sample was collected by cardiac puncture at the twelfth week after the surgical procedure, during euthanasia. Urea and creatinine were determined using a Bioclin bs-120 biochemical analyzer.

Graph Prism® software was used for the statistical analysis of the results, which were expressed as mean and/or standard deviation of the mean (SD) and analyzed using the Student's T-test, $p \leq 0.05$ was considered significant.

Results and Discussion

The surgical procedures of total nephrectomy and contralateral partial nephrectomy were performed in a single surgical phase, not promoting postoperative complications or difficulties for the surgeon as reported by Gava., *et al*. [22]. Considering the cost of making the model, performing the nephrectomies at the same surgical time promotes material savings and celerity in the experiment.

During the transoperative period, to reduce the bleeding mentioned by Boudet., *et al*. [20] and by Kumano., *et al*. [13] as a disadvantage of the surgical method to build the experimental model, the cranial and caudal poles of the kidney were ressected by crushing with a Kelly hemostatic forceps, reducing blood loss. Additionally, the Hemospon® hemostatic sponge was used as a hemostatic agent. According to Gabrielli., *et al*. [23], this agent produces an intense and persistent inflammatory response, having healing potential in addition to hemostatic potential. The use of the fibrin sponge helped in hemostasis, but the difference was given by the clamping performed with the hemostatic forceps, because after its removal, bleeding was minimal and the use of a single sponge promoted total hemostasis.

After the procedure, in the immediate postoperative period, all animals showed anesthetic recovery, with some displaying behavioral changes and prostration. Five animals from 5/6 Nx group and one from SHAM group died within the first 24 hours after surgery. After the death of the animals, seven rats from the 5/6 Nx group and eight from SHAM group remained. The measurements were carried out in the surviving animals from each group. Bao., *et al*. [6] reported that high mortality and little remaining renal mass are the greatest challenges for the 5/6 Nx model. In our experiment, survival rates were approximately 59% for the animals in the 5/6 Nx group and 89% for the rats in the SHAM group in the first 24 h. Teles., *et al*. [24] reported survival of only 41% of the 5/6 Nx animals which did not receive any type of treatment. The higher rates of postoperative success were evidenced by the reduction in animal mortality when the technique of crushing the renal poles was adopted. This fact suggests that the reduction in hemorrhage resulting from renal resection allowed a better recovery of the remaining renal parenchyma, being one of the factors responsible for the lower use of animals in the surgical technique for making the 5/6 Nx model.

The animals in the 5/6 Nx group showed a lower mean body weight than the SHAM group. The difference between the two groups was significant ($P = 0.0029$), but in both groups the weight evolution was positive during the first 11 weeks of evaluation (Figure 2). Yin., *et al*. [21]and Hamzaoui., *et al*. [19] also observed a lower body weight in the nephrectomized group. Koppe., *et al*. [25] reported that besides body weight losses, homeostatic energy and protein balance disorders are characteristics of many diseases, including CKD.

Systolic blood pressure was measured in animals from both groups from the fifth postoperative week onwards, and higher values were observed in the 5/6 Nx group (Figure 3), with a significant

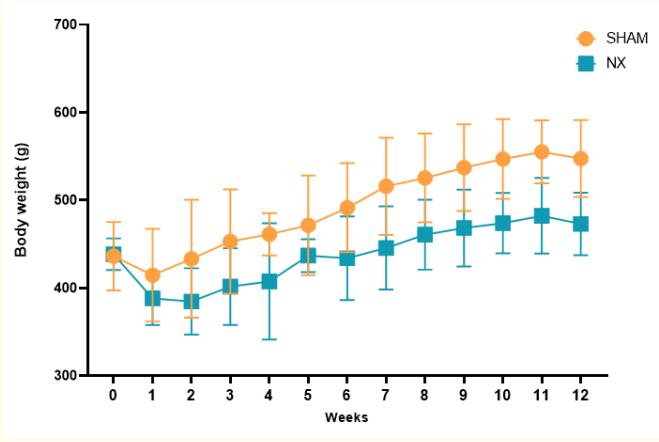

Figure 2: Evolution of body weight in rats of the SHAM and 5/6 Nx groups over 12 weeks evaluated by Student's T-test (P = 0.0029). UFF, 2019.

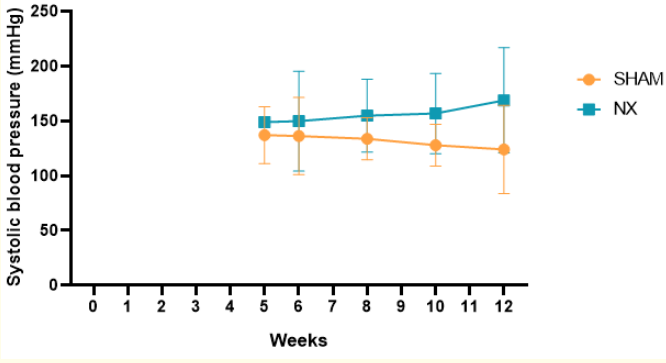

Figure 3: Comparison of mean systolic blood pressure in rats of the SHAM and 5/6 Nx groups evaluated by Student's T-test (P = 0.0006), UFF, 2019.

difference between groups (P = 0.0006). As stated by Ergür., *et al.* [18], hypertension is a common change seen in patients with CKD. Kosaka., *et al.* [26] reported that experimental models of nephrectomy in rats induce an increase in blood pressure and are therefore widely used to elucidate this change in humans. The results found were like those described by Gava., *et al.* [22] and Ergür., *et al.* [18] in their works wih 5/6 Nx model.

When evaluating urine, one of the analyzed parameters was urinary volume, which was higher in the 5/6 Nx than in the SHAM rats (Figure 4), as also reported by Gava., *et al.* [22] and Hamzaoui., *et al.* [19]. Bartlett., *et al.* [27] considered polyuria as one of the clinical signs of CKD in animals. The evaluation was conducted in two moments: at the fifth postoperative week, when the difference between the groups was significant (P < 0.0001), and at the twelfth week, when it was not significant (P = 0.051691).

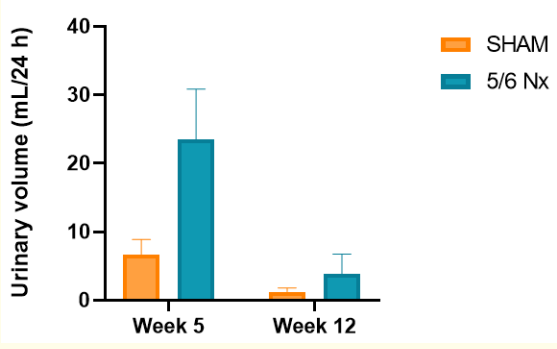

Figure 4: Mean 24-h urinary volume in rats of the SHAM and 5/6 Nx groups at the fifth- and twelfth-weeks post-surgery evaluated by Student's T-test, week 5 (P = 0.000227), week 12 (P = 0.051691), UFF, 2019.

Total urinary protein was higher in the animals of the 5/6 group (Figure 5) in the two weeks of evaluation. He., *et al.* [14] and Yin., *et al.* [21] also found higher proteinuria in animals that underwent 5/6 nephrectomy compared with the control group. This assessment was performed at two different times. In the first measurement, at five weeks, there was no significant difference between the groups (P = 0.1316), whereas in the second, at 12 weeks, a significant difference was detected (P < 0.0001).

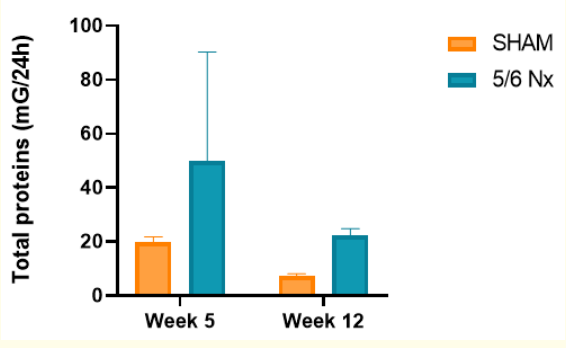

Figure 5: Twenty-four-hour urinary protein in rats of the SHAM and 5/6 Nx groups at the fifth- and twelfth-weeks post-surgery evaluated by Student's T-test, week 5 (P = 0.1316), week 12 (P < 0.0001), UFF, 2019.

At the twelfth week after the surgical procedures, blood was collected by puncturing the lateral tail vein of the animals of both groups. Urea analysis revealed a serum increase of this analyte in the animals of the 5/6 Nx group compared with those of the SHAM group ($P < 0.0001$) (Figure 6). This alteration is in agreement with those described by He., *et al*. [14] and Gava., *et al*. [22], who compared the 5/6 nephrectomy and control groups.

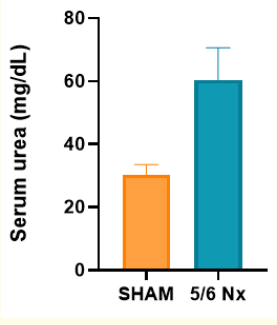

Figure 6: Comparison of serum urea in the SHAM and 5/6 Nx groups at the twelfth postoperative week evaluated by Student's T-test ($P < 0.0001$), UFF, 2019.

Like urea, serum creatinine was higher in the animals of the 5/6 Nx group than in the control group (Figure 7), and the difference between the two groups was significant ($P < 0.0001$). According to Huidobro., *et al*. [28], creatinine is established as the biomarker of choice for evaluating glomerular filtration. He., *et al*. [14] and Hamzaoui., *et al*. [19] obtained equivalent results.

As we observed in the evaluated parameters, the methodology used to obtain the 5/6 Nx experimental model was effective, because although some parameters normalize over time, this compensation is expected in patients with CDK, but it is worth nothing that under stress, this patient may decompensate, further aggravating his condition.

Conclusions

Five-sixths nephrectomy has been consolidated as one of the methods for creating the experimental model of chronic kidney disease. To reduce the complications of this method, we made use of maneuvers to facilitate the technique and reduce the bleeding present in the transoperative period, with good results. The use of hemostatic compression performed with forceps, associated with the application of the Hemospon® sponge, reduced morbidity and mortality in the preparation of the Nx 5/6 experimental model. The models used fit the established parameters of chronic kidney disease and renal failure, confirming the effectiveness of the method. All techniques for creating this model are highly aggressive to the animal, so it is important to continuously search for ways to reduce the impact of surgical stress.

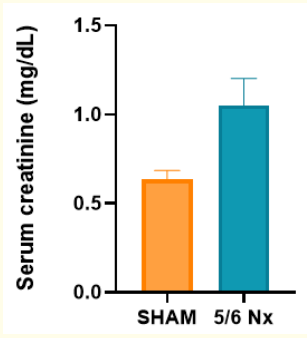

Figure 7: Comparison of serum creatinine in the SHAM and 5/6 Nx groups at the twelfth postoperative week evaluated by Student's T-test ($P < 0.0001$), UFF, 2019.

Acknowledgements

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of the Brazilian Ministry of Education.

Bibliography

1. National Kidney Foundation. "K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification". *American Journal of Kidney Diseases: The Official Journal of the National Kidney Foundation* 39.2 (2002): S1-266.

2. Bargman JM and Skorecki K. "Chronic kidney disease". In: Longo D *et al.* (Eds.). *Harrison's Principles of Internal Medicine*. 18. ed. New York: McGraw-Hill Education (2011): 2308-2321.

3. Jha V., *et al.* "Chronic kidney disease: Global dimension and perspectives". *The Lancet* 382.9888 (2013): 260-272.

4. Lv JC and Zhang LX. "Prevalence and Disease Burden of Chronic Kidney Disease". *Advances in Experimental Medicine and Biology* 1165 (2019): 3-15.

5. Câmara NOS., *et al*. "Kidney disease and obesity: Epidemiology, mechanisms and treatment". *Nature Reviews Nephrology* 13. 3 (2017): 181-190.

6. Bao YW., *et al.* "Kidney disease models: tools to identify mechanisms and potential therapeutic targets". *Zoological Research* 39.2 (2018): 72-86.

7. Wei Q and Dong Z. "Mouse model of ischemic acute kidney injury: Technical notes and tricks". *American Journal of Physiology - Renal Physiology* 303.11(2012): 54-76.

8. Pearce RM. "The influence of the reduction of kidney substance upon nitrogenous metabolism". *The Journal of Experimental Medicine* 10.5 (1908): 632-644.

9. Bradford JR. "The results following partial Nephrectomy and the influence of the Kidney on Metabolism". *The Journal of Physiology* 23.6 (1899): 415-496.

10. Macnider WD. "The development of the chronic nephritis induced in the dog by uranium nitrate. A functional and pathological study with observations on the formation of urine by the altered kidneys". *Journal of Experimental Medicine* 49.3 (1929): 387-410.

11. Heymann W., *et al.* "Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions". *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, N.Y.)* 100.4 (1959):660-664.

12. Allison ME., *et al*. "Pathophysiology of experimental glomerulonephritis in rats". *Journal of Clinical Investigation* 53.5 (1974):1402-1423.

13. Kumano K., *et al.* "A new method of inducing experimental chronic renal failure by cryosurgery". *Kidney International* 30.3 (1986): 433-436.

14. He J., *et al.* "Bone marrow stem cells-derived microvesicles protect against renal injury in the mouse remnant kidney model". *Nephrology* 17.5 (2012): 493-500.

15. Chanutin A and Ferris JR. "Experimental renal insufficiency produced by partial nephrectomy: I. control diet". *Archives of Internal Medicine* 49.5 (1932):767-787.

16. Platt R., *et al.* "Experimental renal failure". *Clinical Science* 11.3 (1952): 217-231.

17. Avioli LV., *et al.* "Intestinal Calcium Absorption: Nature of Defect in Chronic Renal Disease". *Science* 166.3909 (1969) :1154 LP - 1156.

18. Ergür BU., *et al.* "The effects of α-lipoic acid on aortic injury and hypertension in the rat remnant kidney (5/6 nephrectomy) model". *Anatolian Journal of Cardiology* 15.6 (2015): 443-449.

19. Hamzaoui M., *et al.* "5/6 nephrectomy induces different renal, cardiac and vascular consequences in 129/Sv and C57BL/6JRj mice". *Scientific Reports* 10.1 (2020):1-9.

20. Boudet J., *et al.* "Experimental chronic renal failure in the rat by electrocoagulation of the renal cortex". *Kidney International* 14.1 (1978): 82-86.

21. Yin J., *et al.* "Renalase attenuates hypertension, renal injury and cardiac remodelling in rats with subtotal nephrectomy". *Journal of Cellular and Molecular Medicine* 20.6 (2016):1106-1117.

22. Gava AL., *et al.* "Effects of 5/6 nephrectomy on renal function and blood pressure in mice". *International Journal of Physiology, Pathophysiology and Pharmacology* 4.3 (2012):167-173.

23. Gabrielli MA., *et al.* "Avaliação histológica de agentes hemostáticos implantados em mandíbulas de coelhos Histologic Evaluation of Hemostatic Agents in the Rabbit Mandible". *Revista de Cirurgia e Traumatologia Buco-Maxilo-Facial* 9.2 (2009): 97-106.

24. Teles F., *et al.* "Brazilian red propolis attenuates hypertension and renal damage in 5/6 renal ablation model". *PLoS ONE* 10.1 (2015): 1-15.

25. Koppe L., *et al.* "Kidney cachexia or protein-energy wasting in chronic kidney disease: facts and numbers". *Journal of Cachexia, Sarcopenia and Muscle* 10.3 (2019): 479-484.

26. Kosaka S., *et al.* "Effects of angiotensin II AT1-receptor blockade on high fat diet-induced vascular oxidative stress and endothelial dysfunction in dahl salt-sensitive rats". *Journal of Pharmacological Sciences* 121.2 (2013): 95-102.

27. Bartlett PC., *et al.* "Case-control study of risk factors associated with feline and canine chronic kidney disease". *Veterinary Medicine International* 2.10 (2010): 34-46.

28. Huidobro EJP., *et al*. "Estimation of glomerular filtration rate with creatinine". *Revista Medica de Chile* 146.3 (2018): 344-350.