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Effect of Nephrectomy on Some Haemato-Biochemical Parameters in Adult White Male New Zealand Rabbits Strain

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Abstract

THE CURRENT STUDY aimed to evaluate the effect of removing the left kidney on renal functions and some hematological variables in white male New Zealand rabbits. This study enrolled randomly on 60 rabbits which allocated in four equal groups 15 of each. Total nephrectomy was performed aseptically for all rabbits and then general health, clinical, and biochemical hematological investigations were taken one month after nephrectomy, then two months, and three months later. The results showed a significant decrease in the number of red blood cells, hemoglobin concentration, white blood cells, and Packed Cells Volume P.C.V. % one month after the operation, then these variables began to rise after two and then three months, compared with the control group, and at the level of probability ($P \leq 0.05$). While biochemical analysis for kidney function showed a significant increase in the concentrations of uric acid, urea, and creatinine, in the group of rabbits one month after the nephrectomy compared to the control group, while the level of these variables began to decrease after two months, up to the third month, but this decline did not reach the level of the control group, and at the level of probability ($P \leq 0.05$). Conclusion: in this research, it has been found that the unilateral nephrectomy in white male New Zealand rabbits leads to physiological and biochemical changes that vary between decrease and increase depending on over different time periods one, tow, and three months after total nephrectomy.

Keywords: Nephrectomy, Haematology, Renal Function, Rabbits.

Introduction

The kidney is a crucial organ of the urinary system that helps to maintain homeostasis through a multi-step process that includes filtration, reabsorption, and excretion of waste product. Additionally, it controls the body's fluid and electrolyte balance, as well as the location of renin and erythropoietin production, which regulate blood pressure and erythrocyte production, respectively [1].

Each kidney has millions of microscopic uriniferous tubules that consist of nephrons and collective ducts. The nephron is comprised of a glomerulus and a complex tubular system. The glomerulus and the first portion of the tubular system, known as the proximal convoluted tubule (PCT), are located in the renal cortex. Following the (PCT), the loop of Henle, a hairpin-like structure, penetrates the medulla and returns to the cortex to connect with the distal convoluted tubule (DCT). Finally, the nephron drains into the collecting duct via connecting tubules [2].

There are a large number of kidney disease problems, which ultimately raise the need for partial or complete removal of the damaged kidney in order to save the patient's life, this is because damaged kidneys cannot adequately clean the blood of some toxins, there is also a change in the concentration of blood components that are normally regulated by the kidneys [3]. Therefore, surgical management is the treatment of choice and has developed profoundly in the past decade [4]. In additionو nephrectomy is a technique that requires complete surgical removal of the kidney, is the gold standard treatment for many kidney diseases while preserving the other kidney normally [5]. This procedure involves removing only the kidney itself, and does not involve surrounding tissues or structures. It includes separating the kidney from the ureter, blood vessels, and surrounding connective tissue, and nearby lymph nodes and adrenal glands are not removed [6]. The development of new surgical technologies and their translation from research to the clinical setting require appropriate experimental models [7]. In modern

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human medicine, the New Zealand white rabbit is an appropriate animal model for anatomical and histological studies because it is calm and tolerant of disorders better than other species [8]. Besides this, nephrectomy is most commonly performed on healthy rabbits for research purposes because it serves as a model for human renal damage and kidney transplantation [9], so a rabbit kidney was used for this study because several important parameters can be evaluated, where tissue morphology and composition can be studied qualitatively and quantitatively [10]. After nephrectomy, the process of compensation by the remaining kidney leads to structural hypertrophy and functional hyperfiltration in the glomeruli, regarding compensatory structural adaptations, some studies have shown that the degree of structural hypertrophy in the remnant kidney is significantly correlated with postoperative and recovery of renal function [11]. Renal damage is evaluated using uric acid, urea, and creatinine in the blood. Creatinine is a recognized marker for measuring glomerular filtration rate (GFR), a diagnostic parameter for measuring and evaluating renal function [12]. Additionally, the blood parameters primarily provide information on necrosis, inflammation, many visceral organ infections, and stress factors [13]. The present study was carried out to evaluate the effects of nephrectomy on the body in terms of biochemical investigations and blood components test and complete blood count (CBC).

Material and Methods

Study Area

The study was conducted in the biology department/college of Science/Mosul University, Iraq.

Animals

The study was performed in 60 healthy adults, local breed male rabbits Oryctolagus cuniculus, New Zealand white Rabbits, Obtained from local markets, aged (9-12) months, weighting (1250-1500) g. Each rabbit underwent a physical examination, and any that were ill were excluded from the study (feverish, with nasal or ocular discharge, diarrhea, ear mites, unusual lung sounds, or significant wounds). The rabbits were housed in wooden cages with bedding that complied with present regulations. The cages were located in an enclosed room with forced air, a water supply, and nibble drinkers. The animals were subjected to (10 hours of darkness and 14 hours of light). The humidity was regulated between 60 and 70% and the typical temperature was between 25 and 28 °C. The animals had permanent access to water and granulated, green fodder and were under constant veterinary care.

Animals undertaken same condition of health, accommodation, feeding and monitoring. Ivermectin 1% at a dose of 0.2 mg/ kg body weight subcutaneously was used for this purpose [14].

Ethical Approval

The study had been approved by the Institutional Animal Care and Use Committee of the University of Mosul. College of Veterinary Medicine (Ref: UM. VET. 2023.020 / Date: 17-3-2023).

Experimental design

Experimental animals were divided randomly into four equal groups: Group A (time zero) as a control group and three groups of equal treatment (B, C, D). All three groups B, C and D were removed from the left kidney at the same time. Then euthanasia of these animals was performed by decapitation successively, after 1, 2 and 3 months. Blood samples were collected from all groups before euthanasia to detect and assess renal function and blood pictures.

Surgical techniques

Rabbit undergoing protocol of general anesthesia including a mixture of Ketamine hydrochloride 10% (Rotexmedica, Germany) 35 mg/kg body weight and Xylazine hydrochloride 2% (Interchemie, Holland) 5 mg/kg body weight. Intramuscularly, the dose is repeated if necessary [15]. The site of operation was clipped, shaved and prepared aseptically with 2.5 % tincture of iodine. A five cm full-thickness ventral midline laparotomy incision was made. The left kidney was carefully identified through elevation mesocolon from the abdominal cavity, the left renal artery and vein were identified and ligated by double ligature using absorbable suture material, then the ureter was also ligated separately, cutting done between two ligatures, the kidney exteriorized out of abdominal cavity then the muscle, subcutaneous and skin closed the external suture materials removed 14 days post-surgery [16; 17].

Post-operative Care

After the nephrectomy, animals were housed in a clean and excellent environment for recovery. On the first post-operation day, the animals will be kept only on fluid therapy, on the second and third days; they fed on green grass, and return to their normal fodder. To ensure there were no problems or health issues, all groups were closely watched. In order to avoid infection, animals received intramuscular injections of penicillin and streptomycin at doses of 20.000 I.U. /kg and 10 mg/kg B.W., respectively, for four consecutive days after surgery [18]. Rectal temperature, respiration rate, and heart rate were also tracked every day for five days following surgery.

Samples collection and preparation

For haemato- biochemical studies 7ml of blood samples were collected aseptically from rabbits' heart in the sterilized tube and immediately afterwards 2ml of blood from each animal was delivered into vials containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant for determine of blood hematological parameters which include: Red Blood Cell count (RBCs), Haemoglobin Concentration (Hb), White Blood Cells count (WBCs) and P.C.V.%. The complete haematological examination was performed with a (Micro CC-20Plus analyzer, United States). Remaining 5ml of blood was taken in another sterilized tube without anticoagulant to harvest the serum for estimation of biochemical profiles which include: Urea, Creatinine, Uric acid. Each sample was labeled with the name of the group and assigned a serial number.

Statistical analysis

Using the SAS statistical program [19], the results were evaluated statistically using the simple experimental system in a fully random design. The multiple-range test developed by Duncan was used to assess the differences between the groups, and the outcomes were essential at the probability level (P≤0.05) (20).

Results

Fig. (1), shows a statistically significantly higher the RBCs count in the nephrectomy rabbit group after 3 months compared to the other groups at the level of probability (P≤0.05) with arithmetic mean for it reached (5.79 ± 0.18) million globules/mm3 While the arithmetic mean for the control group (5.69 ± 0.15) million globules/mm3 followed by nephrectomy rabbit group after 2 months with arithmetic mean (4.72 ± 0.14) million globules/mm3 and finally nephrectomy rabbit group after 1 month with arithmetic mean (2.81 ± 0.12) million globules/mm3.

Fig.(2) shows a significant increase in the Haemoglobin Concentration (Hb) in nephrectomy rabbit group after 3 months as compare to the other groups at the level of probability (P≤0.05) with arithmetic mean reached to (12.22 ± 0.06) g/100 ml followed by the control group with arithmetic mean arrived to (9.65 ± 0.37) g/100 ml while the nephrectomy rabbit group after 2 months with arithmetic mean (8. 91 ± 0.04) g/100 ml and the nephrectomy rabbit group after 1 month with arithmetic mean (6.55 ± 0.08) g/100 ml.

Fig.(3) shows a significant increase in the White Blood Cell count (WBCs) in the nephrectomy rabbit group after 3 months But it did not reach the level of control groups with arithmetic mean (4.92 ± 0.04) thousand cells/mm3 while the arithmetic mean of WBCs in control group (6.56 ± 0.24) thousand cell /mm3 and the arithmetic mean of nephrectomy rabbit group after 2 months arrived to (4.12 ± 0.07) thousand cell/mm3 and finally nephrectomy rabbit group after 1 month with arithmetic mean (3.79 ± 0.09) thousand cell /mm3.

Fig.(4) shows a statistically significantly higher %PCV in the nephrectomy rabbit group after 3 months at the level of probability (P≤0.05) compared to the other groups with arithmetic mean reached % (35.35 ± 0.29) While the percentage of the PCV to the control group % (28.48 ± 0.31) followed by the nephrectomy rabbit group after 2 months with arithmetic mean % (25.68 ± 0.17), finally the %PCV in nephrectomy rabbit group after 1 month reached to % (18.57 ± 0.20).

Fig.(5) shows a significant increase in the concentration of uric acid in the nephrectomy rabbit group after 1 month compared to the rest of the groups, with an arithmetic mean of (3.90 ± 0.51) mg/100 ml and at a probability level (P≤0.05), while the arithmetic mean for the Control group (2.34 ± 0.62) mg/100 ml. While the arithmetic mean of nephrectomy rabbit group after 2 months was (3.19 ± 0.10) mg/100 ml, and the arithmetic mean of nephrectomy rabbit group after 3 months increased, but did not reach the level of the control group, with an arithmetic mean of (2.79 ± 0.12) mg/100 ml.

Fig.(6) shows a significant increase in the concentration of urea in the rabbit group one month after nephrectomy, with an arithmetic mean of (38.91 ± 0.53) mg/100 ml and at a probability level (P≤0.05), while the arithmetic mean for the control group reached (21.26 ± 0.76) mg/100 ml. The arithmetic mean of the rabbit group two months after nephrectomy was (35.23 ± 0.61) mg/100 ml, while the urea concentration decreased in the rabbit group three months after resection, but did not reach the level of the control group, with an arithmetic mean of (30.27 ± 0.80) mg/100 ml.

Fig.(7) shows a significant increase in creatine concentration in the rabbit group one month after nephrectomy, with an arithmetic mean of (1.78 ± 0.6) mg/100 ml compared to the rest of the groups, and at the probability level (P≤0.05), while the arithmetic mean for the control group reached (0.92 ± 0.2) mg/100 ml. And the arithmetic mean of the rabbit group two months after the resection was (1.42 ± 0.30) mg/100 ml, the creatine concentration decreased in the rabbit group three months after the resection of the left kidney, with an arithmetic mean of (1.24 ± 0.52) mg/100 ml, but it did not reach to the control group level.

Discussion

The blood parameter is a dependable and essential means used to evaluate and monitor the nutritional and health status of animals [13]. The kidney produces a hormone-like substance called erythropoietin, which is a glycoprotein produced by specialized cells in the kidney in response to relative hypoxia to promote the formation of red blood cells in the bone marrow [21]. In case of removal of one of the kidneys and as a result of loss an amount of blood during the surgery, there will be a decrease in the concentration of this hormone immediately after the operation and up to one month after the operation, therefore there will be a decrease in the number of red blood cells, haemoglobin and the PCV%, considering that haemoglobin is one of the most important basic components of red blood cells because it carries oxygen and transports it through the blood, and this is an important explanation for the decrease RBCs, Hb and PCV% one month after the removal of one of the kidneys [22]. Several studies have indicated a decrease in the number of red blood cells and haemoglobin, and %PCV after removal of the kidney, perhaps due to a deficiency in the secretion of the hormone erythropoietin, which is responsible for preparing the process of forming red blood cells in the bone marrow [23], Lack of the iron element, which is a component of the haemoglobin molecule, is one of the causes of hemoglobin deficiency after kidney removal. This deficiency may result from an inhibition of iron absorption from the intestine shortly after kidney removal [24], this due to effect of tow hormones the hepcidin which is an iron-regulating peptide hormone that produced chiefly by hepatocytes, and the erythropoietin which stimulates erythropoiesis and the production of erythroferrone (ERFE) which inhibits hepcidin [25], at nephrectomy the concentration of erythropoietin and erythroferrone decrease [26] there for the hepcidin concentration increase thereby inhibit iron absorption from intestinal enterocytes [27]. Also, the fall in the concentration of haemoglobin after surgery could be attributed to preoperative fasting, anesthesia, intra-operative bleeding, surgical stress and abdominal inflation [28]. As for the reason for the decrease in the number of white blood cells one month after the removal of the kidney, it may be due to the exposure of white blood cells to programmed death (Apoptosis) immediately after the nephrectomy, or destroyed and dissolved these cells in the bloodstream, then there may be an accumulation in showing the effect at the meeting of the two mechanisms [29]. And there is no increase in the number of white blood cells immediately after resection because that linked to the occurrence of infection or inflammation in the body as an important part of its natural defense mechanism, however, in the case of nephrectomy, the white blood cells will decrease and then gradually rise until they reach to normal level [30].The WBC count in rabbits may vary dramatically as a result of circadian rhythms (diurnal fluctuations and variation within a month), nutritional status and dietary differences, and differences in age, gender, and breed [31].

As for the reason for the high numbers of red blood cells, haemoglobin and %PCV after three months as a result of the operation (nephrectomy), it may be due bone marrow stimulation occurs by increasing the secretion of erythropoietin from the remaining kidney by up to double which mean the residual kidney does a double job to compensate for the loss of the kidney, where the secretion of the hormone erythropoietin increases, which stimulates the bone marrow to increase the production and formation of red blood cells, haemoglobin, and PCV% [22;13]. As for the reason for the high number of white blood cells three months after the removal of the kidney, this may be a sign of developing infection in the early postoperative period, it may also be part of a normal surgical response [32]. On the other hand, the results of the current study showed an increase in the concentration of uric acid in male rabbits one month after the removal of the left kidney, as uric acid is a chemical substance produced when the body breaks down purines. Normally, purines are produced in the body [33]. Where most of the uric acid dissolves in the blood and moves to the kidneys, after which it is excreted through urine. The reason for high uric acid may be due to an increase in the activity of the enzyme Xanthene Oxidase, which increases the formation of uric acid [34] and a decrease in the rate of purification of the remaining kidney or may be due to increased reabsorption of uric acid from the distal tubules in the remaining kidney [35]. As for the reason for the decrease in the concentration of uric acid two months after the removal of the left kidney, reaching the lowest level after three months, it may be due to an adaptation in the remaining kidney cells and an increase in their activity, which leads to a decrease in the effectiveness of the enzyme Xanthene Oxidase, which has a direct relationship with the formation of uric acid, which It leads to a decrease in its concentration in blood serum [22]. Additionally, the results of the current study showed a significant increase in urea concentration one month after nephrectomy, that led to a decrease in the effectiveness of the Insulin hormone or a lack of its secretion, which leads to the loss of the direct energy source represented by glucose, and this leads to the use of alternative energy sources such as fats and proteins, the metabolism of which produces large quantities of urea as a byproduct [36]. Kidneys are the leading organs involved in insulin clearance from the systemic circulation after the liver, they contribute to endogenous glucose production through gluconeogenesis and they are affect insulin and glucose levels in the blood through their contribution to insulin metabolism by filtering it from the blood, thus affecting the concentration of insulin in the blood circulation and also contributing to regulating blood glucose levels throw reabsorbing glucose by proximal convoluted tubule or excreting it in the urine, as needed [37].

As for the reason for the decrease in the concentration of urea in the blood serum from the second month until the third month after resection, it may be due to the occurrence of a state of stability and adaptation of the remaining kidney. We call these phenotypic changes compensatory hypertrophy, which are linked to many functional changes in the remaining kidney, and this is what was indicated by [38]. And the results of our study showed a significant increase in the creatinine concentration in the blood serum of male rabbits one month after the nephrectomy. The reason for the increase may be due to a functional disorder in the cells of the inner layer of the glomerular capillary blood vessels, which leads to an increase in its concentration in the blood serum and a decrease in its excretion in the urine [39]. While the reason for the decrease in creatinine concentration in male nephrectomy rabbits after a month of resection, reaching the concentration of the control group or slightly less in the third month, may be due to an adaptation occurring in the cells of the inner layer of the glomerular capillary blood vessels, increasing their activity and division, and increasing their expansion, given that kidney cells are dilated cells, which It leads to an increase in its excretion in the urine and a decrease in its concentration in the blood serum [22]. In addition, serum creatinine is inversely correlates with glomerular filtration rate (GFR) and is a good indicator of renal function as also reported by [3].

Conclusion

In this research, it has been found that nephrectomy in male New Zealand rabbits leads to physiological changes that differ compared to the different periods in which blood components such as RBCs, Hb, WBCs and %PCV decrease one month after nephrectomy while same components rise three months after nephrectomy. In other side the renal function concentrations raised in the rabbit group one month after nephrectomy while it decreased in the other periods.

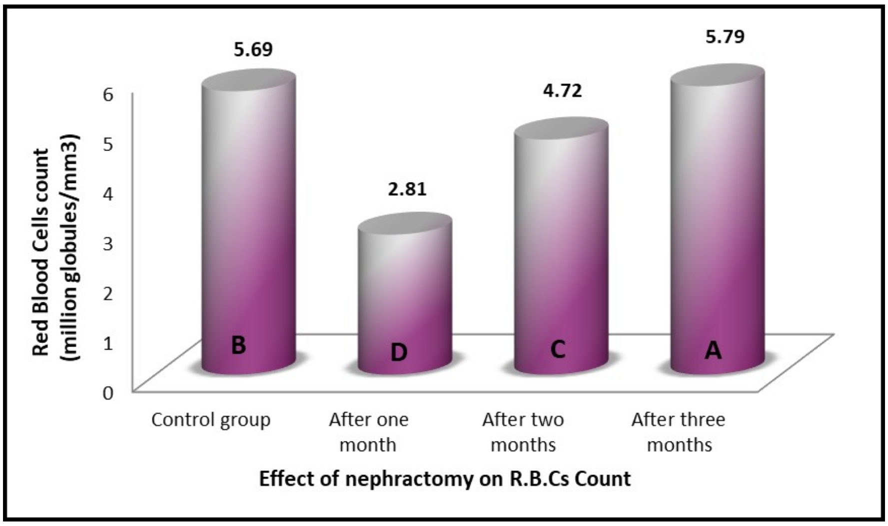

Fig.1. Shows the effect of nephrectomy on RBCs count in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the probability level (P≤0.05), shapes and various letters show a significant difference.

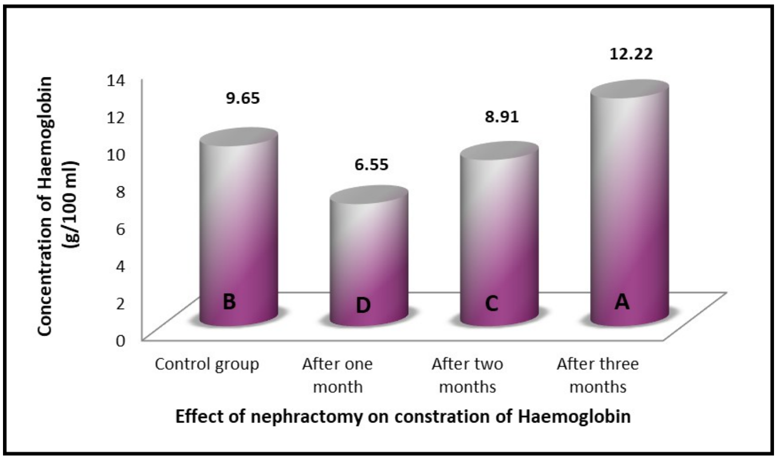

Fig.2. Shows the effect of nephrectomy on the Haemoglobin concentration in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the probability level (P≤0.05), shapes and various letters show a significant difference.

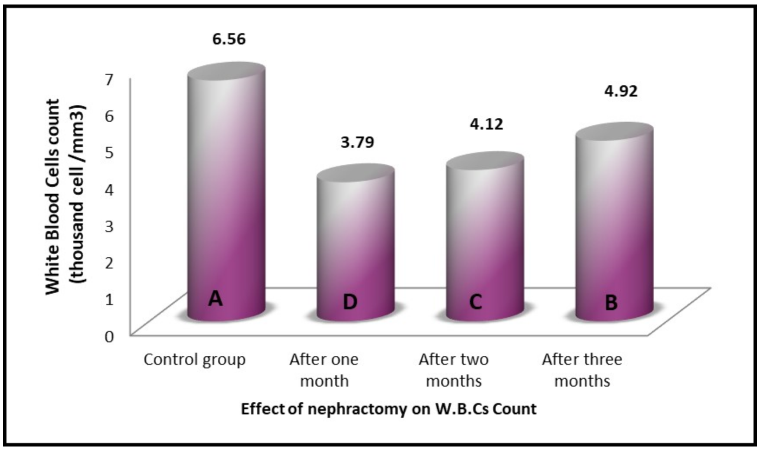

Fig. 3. Shows the effect of nephrectomy on WBCs count in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the confidence level (P≤0.05), shapes and various letters indicate an important variation.

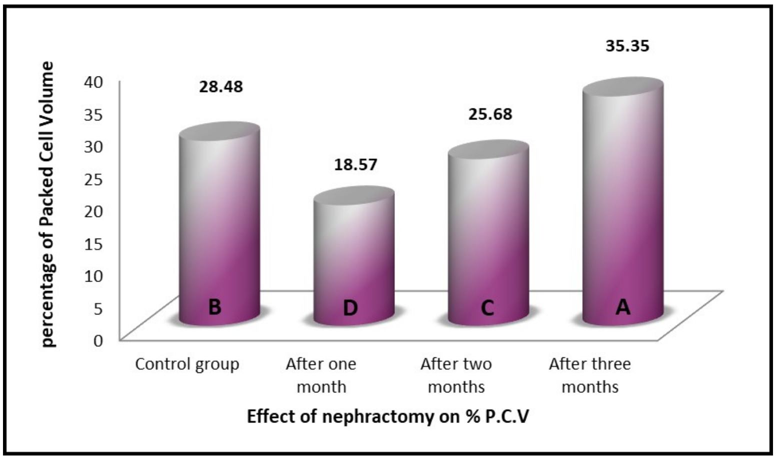

Fig. 4. Shows the effect of nephrectomy on the percentage of PCV in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the confidence level (P≤0.05), shapes and various letters suggest a significant difference.

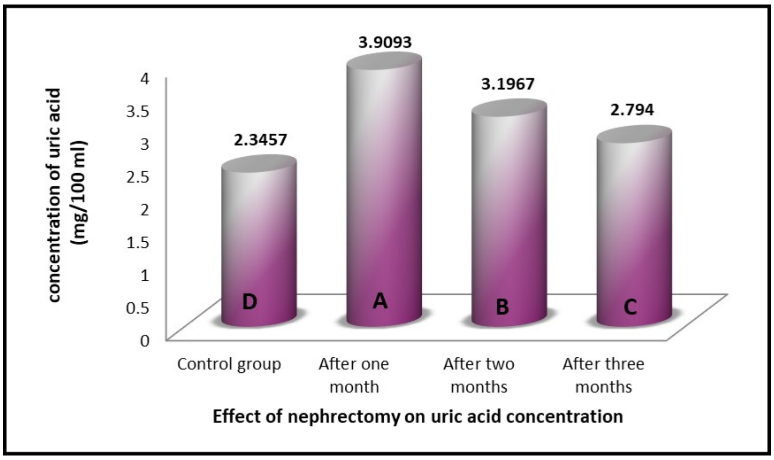

Fig. 5. Shows the effect of nephrectomy on the uric acid concentration in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the confidence level (P≤0.05), shapes and various letters suggest a significant difference.

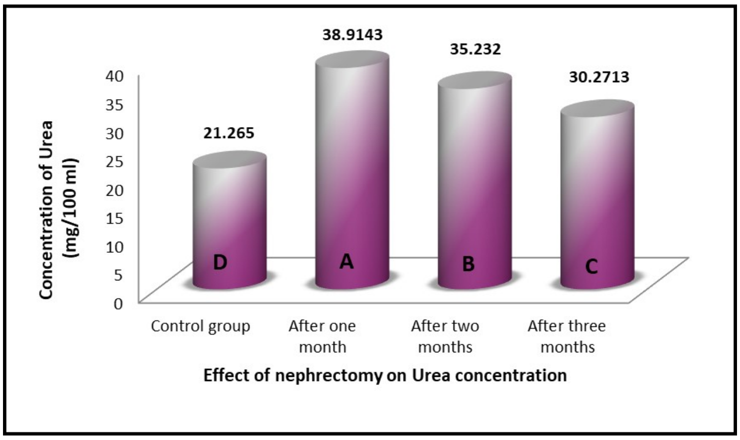

Fig. 6. Shows the effect of nephrectomy on the urea concentration in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the confidence level (P≤0.05), shapes and various letters suggest a significant difference.

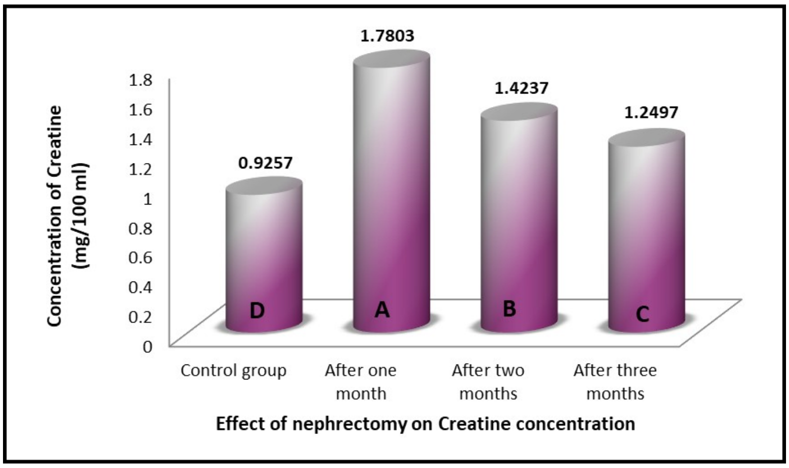

Fig. 7. Shows the effect of nephrectomy on the creatine concentration in multiple periods.

There are 60 rabbits, and values are presented as mean standard deviation. At the confidence level (P≤0.05), shapes and various letters suggest a significant difference.

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تأثير استئصال الكلية على بعض الصفات الدموية-الكيموحيوية في ذكور الأرانب البيضاء البالغة

‘‘New Zealand strain″

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المستخلص

هدفت الدراسة الحالية إلى تقييم تأثير إزالة الكلية اليسرى على وظائف الكلى وبعض المتغيرات الدموية في ذكور الأرانب النيوزلندية البيضاء. أجريت هذه الدراسة عشوائياً على 60 أرنباً وزعت على أربع مجموعات متساوية 15 أرنباً من كل مجموعة. تم إجراء استئصال الكلية بشكل معقم لجميع الأرانب ثم تم إجراء فحوصات الدم الصحية العامة والسريرية والكيميائية الحيوية بعد شهر واحد من استئصال الكلية، ثم بعد شهرين، وبعد ثلاثة أشهر. أظهرت النتائج انخفاضا معنويا في عدد خلايا الدم الحمراء وتركيز الهيموجلوبين وخلايا الدم البيضاء وحجم الخلايا المرزومة (P.C.V.) % بعد شهر من العملية، ثم بدأت هذه المتغيرات في الارتفاع بعد شهرين ثم ثلاثة أشهر، مقارنة مع المجموعة الضابطة، وعند مستوى الاحتمال (P≤0.05). بينما أظهر التحليل الكيموحيوي لوظائف الكلى ارتفاعاً معنوياً في تراكيز حمض البوليك واليوريا والكرياتينين لدى مجموعة الأرانب بعد شهر واحد من إجراء عملية استئصال الكلية مقارنة بمجموعة السيطرة، بينما بدأ مستوى هذه المتغيرات في الانخفاض بعد شهرين ، حتى الشهر الثالث، إلا أن هذا الانخفاض لم يصل إلى مستوى مجموعة السيطرة، وعند مستوى الاحتمال (P≤0.05). الاستنتاج: في هذا البحث وجد أن استئصال الكلية من جانب واحد في ذكور الأرانب النيوزلندية البيضاء يؤدي إلى تغيرات فسيولوجية وبيوكيميائية تتراوح بين النقصان والزيادة اعتمادا على فترات زمنية مختلفة بعد شهر وشهرين وثلاثة أشهر من استئصال الكلية الكلي.

الكلمات المفتاحية: استئصال الكلية ؛ الفحوصات الدموية؛ وظائف الكلية؛ الارانب.