

## Protective role of *Punica granatum* juice in inhibit nephrotoxicity Induced by amikacin in albino Rabbits

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### Abstract

The current investigation aimed to evaluate the role of *Punica granatum* juice extract to reduce nephrotoxic formation by amikacin in male and female albino rabbits by measuring some criteria for biochemical functions, such as creatinine, urea, albumin, total protein. Given amikacin (80mg/ kg) daily by injection in muscle for period of 15 days caused a significant increase at the level of ( $p \leq 0.05$ ) in the concentration of creatinine, and urea in the serum compared with the group control, while decreased the concentration of albumin and total protein significantly compared with the control group. The rabbits given orally total dose of 100ml several time of amikacin daily pomegranate juice, a concentration of 40%, concomitantly with the injection in muscle at aldose of (80) mg/kg/day) has recorded a significant decrease in the concentrations of creatinine and urea compared with the group of rabbits treated with amikacin only, and increased significantly the albumin and total protein compared with the control group treated with amikacin only.

### Introduction

Many human diseases have been recognized as being a consequence of free radicals damage [1]. Interest in the role of antioxidants in human health has prompted research in the fields of food science to assess fruit and vegetable antioxidants [2, 3]. The majority of the antioxidant capacity of a fruit or vegetable may be from phenolic compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or  $\beta$ -carotene [4]. These phytochemicals may help to protect cells against the oxidative damage caused by free radicals [5,6]. The antioxidant activity of phenolic compounds is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [7].

Renal failure is accompanied by oxidative stress, which is thought to be caused by enhanced production of reactive oxygen species and impaired antioxidant defense. Renal cell injury may culminate in the cell death, which may occur through necrosis, apoptosis or other pathways. Chemicals in general can initiate toxicity because of their intrinsic reactivity with cellular macromolecules [8]. They may initiate injury indirectly by inducing oxidative stress, which caused by excessive production of reactive oxygen species and it may produce a major alteration of protein and nucleic acid structure, damage to DNA, and destruction of the cells by lipid peroxidation. An imbalance between free radicals and defense mechanism leads to cell damage and several diseases. For this reason, the role of nutrition in health has captured the interest of researchers in antioxidants and their capacity to protect the body from damage induced by oxidative stress [9]. Many plants such as *Punica granatum* L, *Citrus limon* possess antioxidant properties.

Pomegranate, (**Al-Ruemman**), has long been attracted a lot of attention for its medical importance. Pomegranate juice was indeed shown recently to

possess impressive antioxidative properties due to its polyphenolics, tannins and anthocyanins. Also, in healthy humans. Pomegranate juice consumption was shown to possess potent antioxidative capabilities against lipoprotein oxidation, and increased serum total antioxidant status.

Aminoglycosides are potent bactericidal antibiotics; they act particularly against aerobic, Gram-negative bacteria. Amikacin is one of the aminoglycoside, mostly used for treatment of severe, hospital-acquired infections with multidrug resistant Gram negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter*, and *Enterobacter* [10].

Aminoglycoside induced nephro and oto-toxicity, which are the limiting factors for their clinical use, in which the oxygen free radicals have been involved. Aminoglycosides, exert their adverse renal effect by generation of reactive oxygen species. Additionally, it has been demonstrated that aminoglycoside form a complex with mitochondrial  $Fe^{+2}$  to catalyze the formation of free radicals [11]. Increased nephrotoxicity has been reported following concomitant parenteral administration of aminoglycoside antibiotics and cephalosporin. Concomitant cephalosporin may spuriously elevate creatinine determinations [8].

### Material and Methods:

In continuation of our investigation of *Punica granatum* fruit extract [12], male and female rabbit, their age between 8-10 months, weight: 900-1750 gram, which bought from local market. These rabbits kept in closed wooden boxes covered by thin metal of Aluminum, its dimension 40x90x60cm. Floored with wood straws with complete cleaning and used antiseptic every two days, light exposit 12 hour, dark exposit 12 hour, temperature was  $25 \pm 2$  °c. The animals were kept for two weeks to adapt them to their new medium and with no disease contact. Feeding of the rabbits depend mixture of (35% wheat, 34% corn, 20% Soya bean, 10% animal protein, 1%

dried milk). Added to it 50 conservative and antifungal with regulate feeding and water continuously along that period from December 2012 to April 2013.

#### Preparation of Plant Extract:

Pomegranate's juice (250 ml) was obtained from cold pressed fruits (about 2kg fresh fruit weight) collected from local market of Iraq during September 2012, which was then concentrated by simple distillation. The methodologies of **Harbone (1983)** and **Wagner et al (1984)** were adopted to prepare the ethanolic extract of concentrated juice (1:1; v/v). The mixture was shaken for 12h on a shaker with reciprocal mechanism. After ethanol evaporation and concentration of the remaining aqueous solution by freeze drying, the residue was weighed and further dried in desiccators.

#### Experimental Method

**Group 1**-four rabbits were treated with Intra Venous (I.V) of normal saline for 14 days, this group served as control.

**Group 2**-four rabbits were treated by injection in muscle of (80) mg/Kg/day of amikacin for 14 days. This group served as positive control for nephrotoxicity induced by amikacin.

**Group 3**-four rabbits treated with oral total dose of 100ml several time daily , the concentration of 40% pomegranate juice concomitantly by injection in muscle dose of amikacin (80) mg/ kg/ day) for 14 days. This group utilized to investigate the possible protective effect of pomegranate against nephrotoxicity induced by amikacin. All animals were anesthetized by ether and sacrificed after 15 days of treatment

#### The results

##### The histology of the kidney of the controlled group.

Kidneys of group 1(control) showed that the cortex of the kidney was containing glomeruli of normal size and structure surrounded by Bowman's capsule (fig: 1). The cortex also occupied by the proximal convoluted tubules which were formed by epithelial cells of pyramidal form, also there was many distal convoluted tubules of normal form which lined by cuboidal cells, so the lumen of these tubules appeared wider than the proximal convoluted tubules.

The medulla of kidney was formed by the tubules and collecting ducts which were lined by cuboidal epithelial cells (fig:2), the tubules were very thin surrounded by squamous cells which were the thin segments of Heule loop.

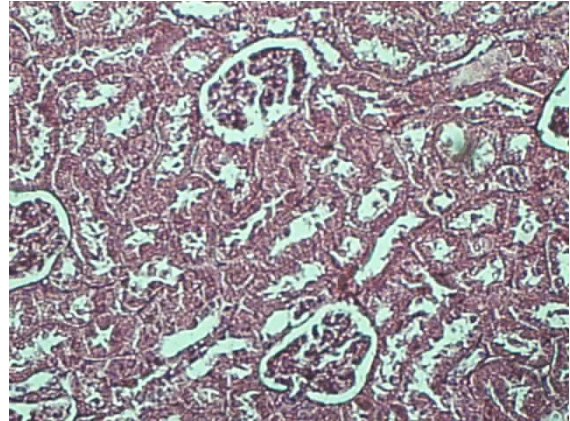


Fig. 1: Section of the kidney show normal Structure appearance of the glomeruli And renal tubules of control group.(H&E x 20)

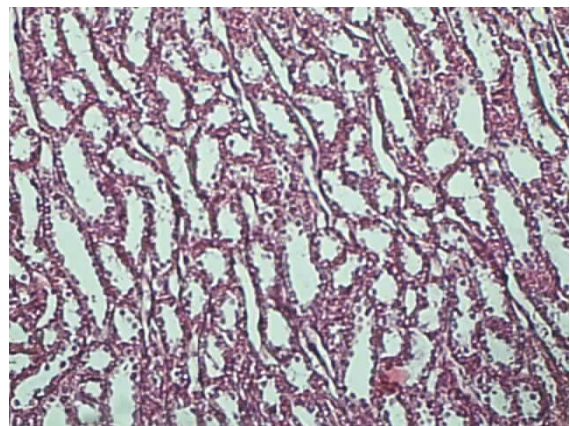


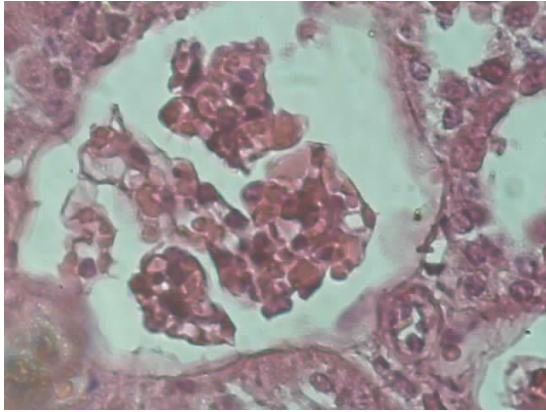
Fig. 2: The medulla of kidney formed by collecting Ducts, tubules and Heule loops (H2E x 20)

#### Nephrotoxic effects of amikacin

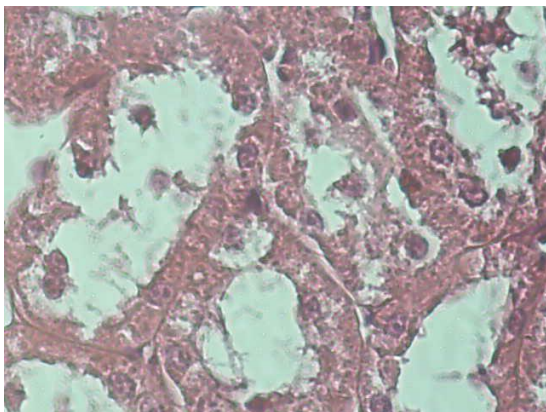
##### Effect of amikacin on the histology of the kidney.

The cortex was containing the glomeruli which were appeared mostly atrophied inside the Bowman's capsule, so the capsular space of bowman was wide (fig: 3a), some of the glomeruli were segmented and broken into many segments. The proximal convoluted tubules was containing a few of desquamated epithelial cells from its wall, whereas the lumen containing a glomerular filtrate and some of degenerated cells (fig: 3b). The distal convoluted tubules revealed normal, appearance.

The renal medulla was containing degenerated cells in it's tubules and collecting ducts, and there were another tubules and collecting ducts of normal structure.



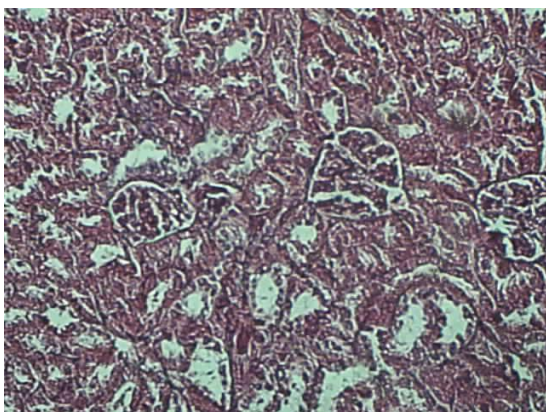
**Fig.3a:** Sections of kidney shows degenerative Changes and necrosis of renal tubules in amikacin treated group.



**Fig. 3b:** Sections of kidney shows degenerative Changes and necrosis of renal tubules in amikacin treated group.

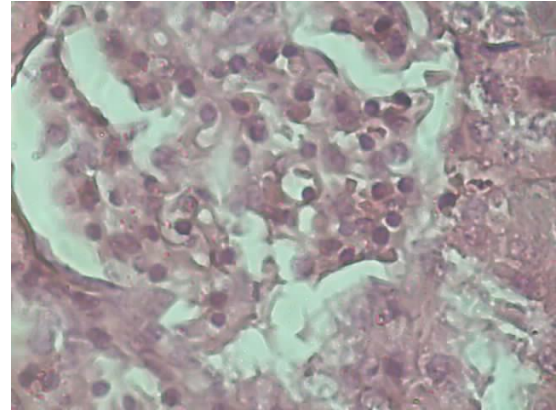
#### **Effects of combination of pomegranate with amikacin on the histology of the kidney**

There were some of the glomeruli which appeared hypertrophied



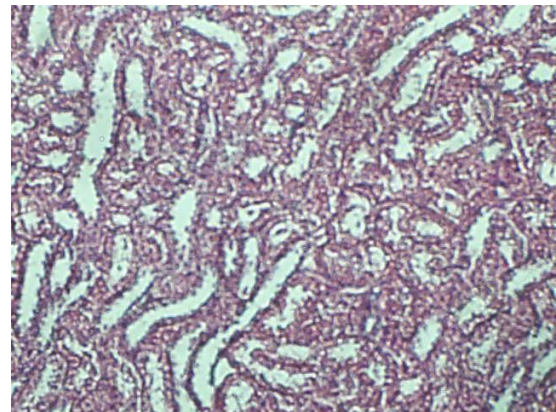
**Fig.4a:** Sections of kidney show slightly degenerative changes with Regeneration of renal tubule in which the appearance look like the normal In amikacin with pomegranate treated group.

Also appeared some of the glomeruli of a atrophied pattern (fig.4b).



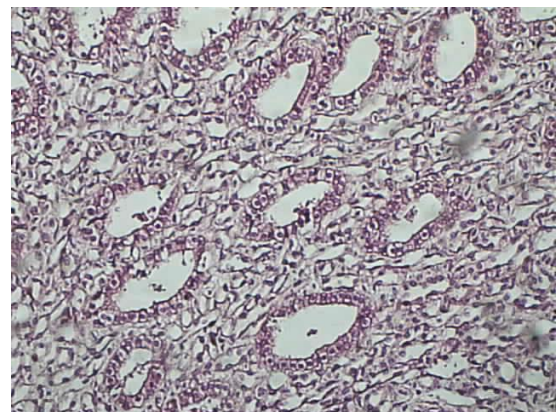
**Fig.4b:** Sections of kidney show slightly degenerative changes with Regeneration of renal tubule in which the appearance look like the normal In amikacin with pomegranate treated group.

The proximal convoluted tubules were of normal structure, also the distal convoluted tubules appeared normal shape and structure (fig: 5), some of the tubules were containing a glomerular filtrate.



**fig.5:** Sections of the renal medulla appeared Convoluted tubules were of normal structure, Also the distal convoluted tubules were Appeared of normal shape and structure (H2E x 20)

The renal medulla appeared of normal structure and shape for the renal tubules and collecting ducts and the interstitial connective tissue was highly infiltrated with different types of cells of connective tissue (fig:6).



**fig.6:** Sections of kidney show the proximal Of normal structure and shape (H2E x 20)

The effect of amikacin on the renal function showed a significant increase ( $p < 0.05$ ) in the serum levels of both creatinine and urea of rabbits treated with 80 mg/kg/ day of amikacin (group 2) compared to the corresponding levels in the control animals of group1, while there was a significant decrease ( $p < 0.05$ ) in the serum levels of both creatinine and urea of rabbits treated with 80 mg/kg/ day of amikacin +100 mg/kg/day of pomegranate juice (group 3) compared to the corresponding levels of rabbits treated with 80 mg/kg/ day of amikacin (group 2). Serum levels for creatinine were ( $2.9 \pm 0.070$  mg/dL and  $1.70 \pm 0.157$  mg/dL) and that for urea were ( $25.4 \pm 0.917$  mg/dL and  $19.220 \pm 0.431$  mg/dL) in group 2 and 3, respectively. (Figures 7 and 8).

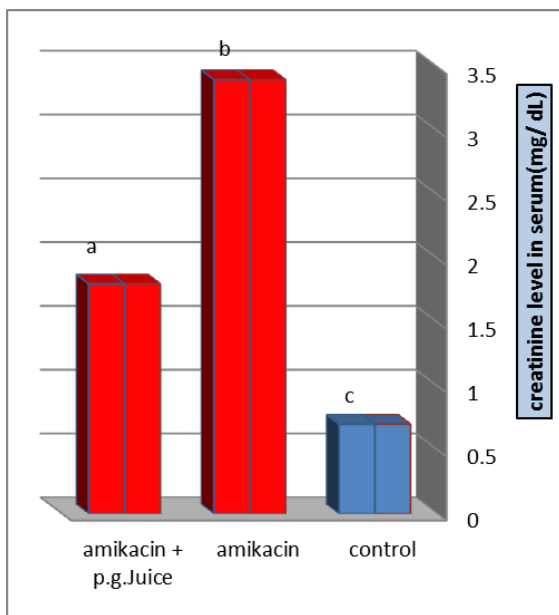


Fig. 7: Effect of amikacin and the combination of P.G With amikacin on the serum Creatinine

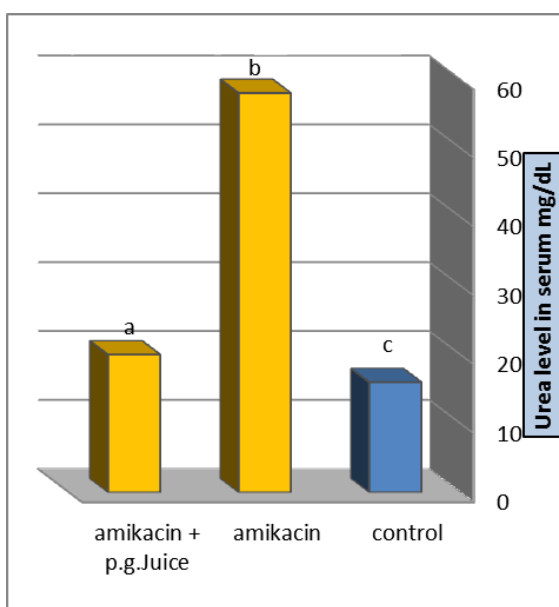


Fig. 8: Effect of amikacin and the combination of P.G With amikacin on the Urea

**Effect of the combination of pomegranate with amikacin on the serum Albumin and total protein concentration:**

There was significant increase ( $p < 0.05$ ) in the serum levels of Albumin of rabbits treated with 80 mg/kg/day of amikacin +100 mg/kg/day of pomegranate (group3) compared to the corresponding levels of rabbits treated with 80 mg/kg/day of amikacin (group 2), the serum levels of albumin were ( $1.598 \pm 0.566$  dL and  $3.683 \pm 0.887$  dL) in group 2 and 3, respectively; while there was a significant decrease ( $p < 0.05$ ) in the serum levels of protein in rabbits treated with 80 mg/kg/ day of amikacin+100 mg/kg/day of pomegranate (group3) compared to the corresponding levels of the rabbits treated with 80 mg/ kg/ day of amikacin respectively (group2). (Figures 9 and 10)

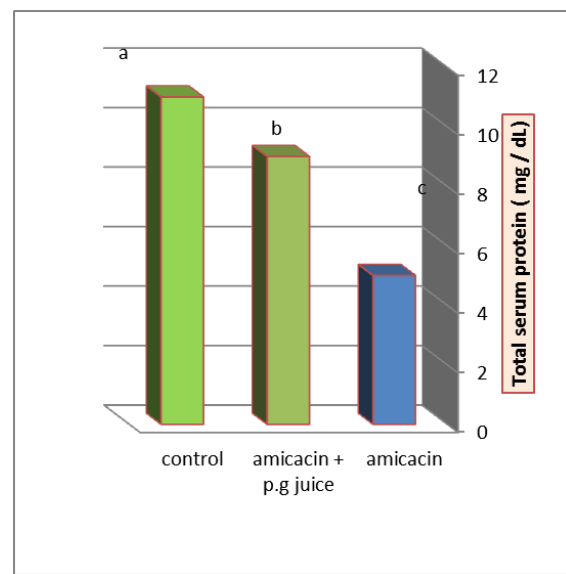


Fig. 9: Effect of amikacin on the serum Protein

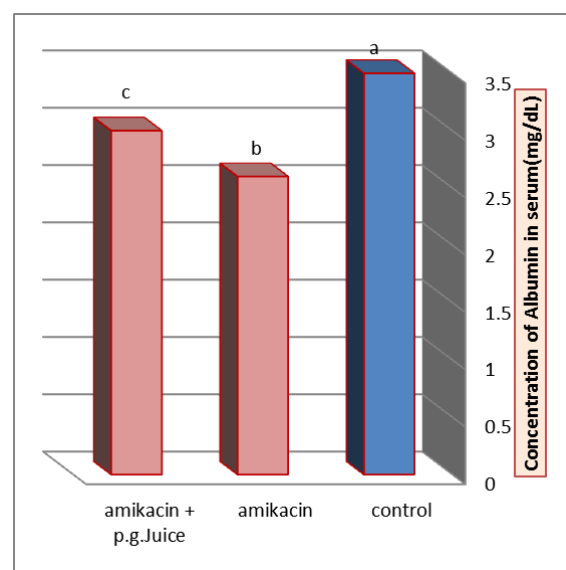


Fig. 10: Effect of Amikacin on the serum albumin

## Discussion

Aminoglycoside antibiotics have long been used as antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic side effects [10]. It has been reported that amikacin may induce free radicals production which implicate a variety of pathological processes [11]. In this study the marked elevation of the levels of both serum creatinine and urea in group 2 compared with group 1 were observed and gave an indication to the reduction in the glomerular filtration. Since serum creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney; therefore such increase of serum creatinine and urea as reported in this study confirm an indication of functional damage of the kidney and these results were consistent with other studies[13]. Also it was found that aminoglycosides cause renal tubular cells undergo necrosis when their cellular Adenosine Tri Phosphate (ATP) stores are severely depleted to a level incompatible with maintenance of basal metabolism and activity of membrane transport pumps [14]. Results of this study showed an improvement in the serum creatinine and urea levels of rabbits treated with combination of pomegranate juice with amikacin (group 3) compared with group 2, and these levels are near the levels in group1. These results are in agreement with results of other study which showed that combination of cimetidine (an inhibitor of cytochrome P450) with gentamicin showed decrease in serum urea and creatinine levels[15]. The elevation of the levels of both serum creatinine and urea in group 2 compared with group1 attributed to the free-radical scavenging properties of the Pomegranate juice, where it help in maintaining the reduction of reduce of both serum creatinine and urea. The antioxidant effects of Pomegranate juice was attributed to its constituents like antioxidant trace elements and flavonoids compounds; therefore Pomegranate juice has been suggested to be able to decrease lipid peroxidation [16]. Also the antioxidant activity of Pomegranate juice is due to phenolic compounds and enzymes (glucose oxidase, catalase and peroxidase)[17]. Results of this study are in

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agreement with results of [18]. which found that natural Pomegranate juice has protective effect against the damage in liver and kidney cells from oxidative stress induced by toxic level of lead in rats[19].

Pomegranate juice in the present study was used as the antioxidant of choice, as it is very rich in polyphenol and demonstrates high capability to scavenge free radicals and to inhibit Low density lipoprotein oxidation in *vitro* and in *vivo*[20]. It have been shown to increase serum antioxidant capacity or decrease oxidative damage of bimolecular [21]. Pomegranate juice has shown significant anti atherosclerotic, anti-hypertensive, antioxidant, and anti-inflammatory effects in human subjects and rabbits models. Pomegranate juice has also been shown to prevent oxidative destruction of nitric oxide and enhance its antioxidant and anti-inflammatory functions.

## Conclusion

Four rabbits were treated by injection in muscle of (80) mg/Kg/day of amikacin for 14 days. This group served as positive control for nephrotoxicity induced by the generation of reactive oxygen species of amikacin. Its effect on the renal function showed significant increase ( $p < 0.05$ ) in the serum levels of both creatinine and urea of group 2 rabbits, compared to the corresponding levels in the control animals of group 1. While there was a significant decrease ( $p < 0.05$ ) in the serum levels of both creatinine and urea of rabbits treated with 80 mg/kg/ day of amikacin + 100 mg/kg/day of pomegranate juice (group 3). These results show that the consumption of pomegranate juice possesses potent antioxidative capabilities against lipoprotein oxidation, and increased serum total antioxidant status, due to its polyphenolics, tannins and anthocyanins.

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## الدور الوقائي لعصير الرمان في الحد من التسمم النفروي nephrotoxicity الناتج عن استخدام المضاد الحيوي الأميكاسين في الأرانب

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### الملخص

هدفت الدراسة الحالية لتقييم دور مستخلص عصير الرمان Punica granatum juice extract للحد من السمية النفرونية Nephrotoxicity المستحدثة بواسطة المضاد الحيوي الأميكاسين amikacin في الأرانب البيضاء من الذكور والإناث من خلال قياس بعض المعايير الكيموحيوية، مثل الكرياتينين واليوريا والألبومين والبروتين الكلي. حيث اعطي الأميكاسين (80 ملغ / كغم) يوميا عن طريق الحقن في العضلات لمدة 15 يوما مما أدى إلى ارتفاع معنوي ( $P \leq 0.05$ ) في مستوى تركيز الكرياتينين ، واليوريا في مصل الدم بالمقارنة مع مجموعة التحكم، في حين انخفض تركيز كل من البروتين الكلي والألبومين بشكل ملحوظ مقارنة مع مجموعة التحكم. أما بالنسبة للأرانب التي تم تجريعها عصير الرمان (100مل) يوميا، وبتركيز 40% لمدة 15 بصورة متزامنة عن طريق الحقن في العضلات بجرعة من الأميكاسين (80 ملغ/ كغ / يوم) سجلت انخفاض معنوي في تركيز الكرياتينين واليوريا مقارنة مع ارناب المجموعة التي تلقت العلاج مع الأميكاسين فقط، وزيادة كبيرة في البروتين الكلي والألبومين بالمقارنة مع مجموعة التحكم التي تم معاملتها بالأميكاسين فقط.