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The Histopathological Effect of Verapamil Against the Nephrotoxicity of Gentamicin in Rats

Hmood Sh. Subhi
Dept. of Pharmacology, Collage of Veterinary Medicine, Tikrit University.
Siham A.Wadee
Dept. of Pharmacology, Collage of Veterinary Medicine, Tikrit University.
Idrees khalaf thamer
Prof. of Histology and Anatomy, Collage of Veterinary Medicine, Tikrit University.

Abstract
Excess amount of gentamicin is a clear cause of nephrotoxicity and the possibility of death, so this study aimed to verify the effect of gentamicin on the tissue sections of the kidneys, and treatment with verapamil to treat the effects caused by gentamicin in the kidneys of rats.
METHODS: A total of 30 rats (3 to 6 months old) raised at high ambient temperature were divided into three equal groups, 10 rats each. The first control group was administered with normal saline, the second group was treated with gentamicin (80 mg/kg body weight by intraperitoneal injection), and the third group was given gentamicin with verapamil at a concentration of 80 mg/kg for gentamicin by intraperitoneal injection at a concentration of 3 mg/kg for verapamil. By oral administration for 30 days.
The samples of the tissue sections of the kidney were taken and all the fatty tissue attached to the organ was removed, as they were placed in a package containing a solution of (10)% formalin for the purpose of histological study of the kidney organ in the study and the activities were evaluated.
Results: In the gentamicin-injected group, the glomeruli belonging to the renal granules showed degeneration and necrosis, and some of them showed dilation, bleeding and atrophy. In the other section, Bowman's area increases, with a sharp expansion of the capsular space, and hemorrhages occur in the epithelial tissue lining the proximal and distal convoluted tubules. Microscopic examination of the gentamicin and verapamil groups indicated the appearance of the kidney cortex in its normal shape with a slight splitting of the capsular space around the glomeruli and the presence of a slight thickening of Bowman's capsule with the nature of epithelial cells in the lumen of the tubules.
Conclusion: treatment with gentamycin results in significant biological changes in a manner that depends on the doses administered to the kidneys. Subsequently, a significant defect in renal tissue appears, compared to the group that included gentamycin and verapamil, where the vast difference between them and the treatment of the defect occurred.
Key words: gentamycin, verapamil, nephrotoxicity, histopathology

Introduction:

Nephrotoxicity is the most serious side effect of gentamycin and occurs in about 15-30% of patients after several days of treatment(1). The nephrotoxicity of gentamicin is due to its accumulation in the distal convoluted tubules and its long-term survival(2). Gentamicin also

accumulates in the renal cortex due to its longer half-life (100 hours versus 30 minutes in plasma(3). It inhibits the Na+,K+ATPase pump and the accumulation of DNA in the distal tubules and produces different types of reactive oxygen species (ROS) associated with increased lipid oxidation, causing nephrotoxicity(4). Studies at the molecular level have shown that after glomerular filtration, gentamicin can diffuse from the lumen of the renal tubules to the basement membrane of the distal tubular cells of the kidney(5). It enters the cell by binding to the negatively charged phospholipids on the basal villi through the process of cellular insertion mediated by megalin-mediated endocytosis Migalin is a receptor protein that is abundantly circulated within the cells of the distal renal tubules(5). It is worth noting that acute tubular necrosis, glomerular destruction, and various types of kidney failure are the main events associated with gentamicin-induced toxicity(6). Slightly elevated serum creatinine is an indication of decreased glomerular filtration rate (GFR), and in gentamicin-induced nephrotoxicity, it peaks in serum during treatment unlike inner ototoxicity(7). Accumulation of gentamicin in the kidneys is a clear indicator of nephrotoxicity (8).

Methods and materials:

1- Preparation of laboratory animal

Forty white rats of both sexes were used in the study, their weights ranged between (300-200) gm and their age ranged from three months to six months. The animals were raised in the animal house of the College of Veterinary Medicine, Tikrit University, on 11/1/2021 until 12/1/2021 and were taken care of under ideal laboratory conditions of lighting, ventilation and appropriate temperature. Three times a week to maintain hygiene, the rats were given water and animal ration designated for them, then they were subjected to the experiment and the work was carried out.

2-Gentamicin preparation

The pure antibiotic gentamicin of Chinese origin was prepared with the following dose (80 mg/kg), which is the focus of the study approved in the current research stage, which is in the form of a powder in a sealed bag away from sunlight, and then the weights of the rats being studied were recorded according to Weight and then determine how much of that dose to be given to each rat.

3-Preparation of verapamil

Verapamil was prepared in the following dose (3 mg/kg) by dissolving it in distilled water, which is the focus of the study approved in the current research stage.

4-For light microscopic examination

The kidneys were extracted, sliced and fixed in 10% formalin solution. The specimens were processed, embedded in paraffin. Specimens were cut at 5 micron thickness sections which were processed for staining with: Hematoxylin and eosin stain for demonstration of the structure of the renal cortex and medlla(9).

5-Statistical evaluation

The data were expressed as mean ± SD. Differences between groups were compared by ANOVA using SPSS software (version 16). A p value less than 0.05 was considered statistically significan(10).

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Results:

The cortex of the kidney contained semi-lobed glomeruli surrounded by capsular preservation and Bowman's capsule. The presence of glomeruli. Also, the tubules found lining a picture of pyramidal cells with acidic cytoplasm around them starvation of the lining champion of simple cubic cells with blood cells in the interstitial tissue shown in the figure (1)

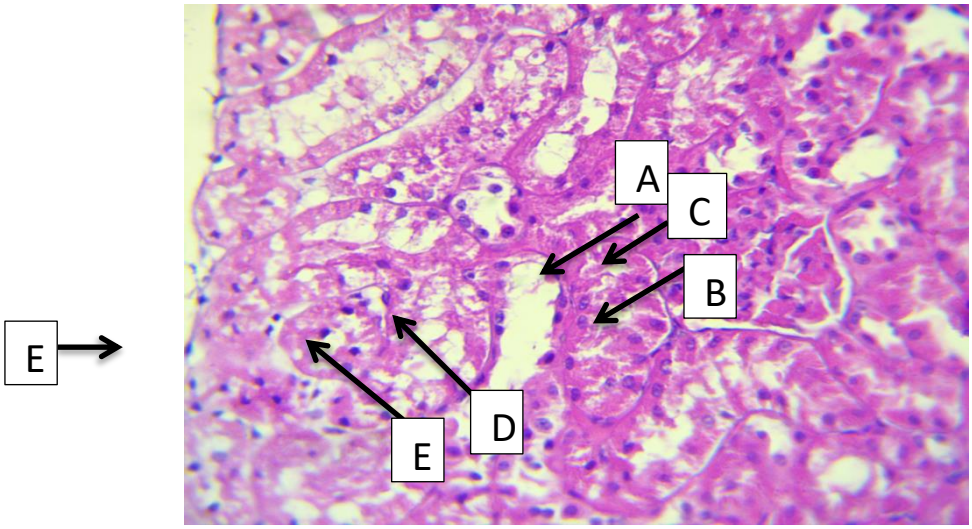

figure (1). renal cortex. Semi-lobular glomerulus (A capsular space (B), Bowman's capsule (C), Proximal convoluted tubules (D), Distal convoluted tubules (E), Leukocytes (F). (H&EX40)

As for the pulp of the other end of the kidneys, the tubules and lining of the renal ducts were found in it of cubic cells with faint cytoplasm, spherical in shape, wide cavities extending longitudinally towards the renal pelvis, and the thin fragments of Henley's loops, in addition to the presence of infiltration in a number of white blood cells and macrophages in the interstitial tissue between the ducts, as shown in the figure (2)

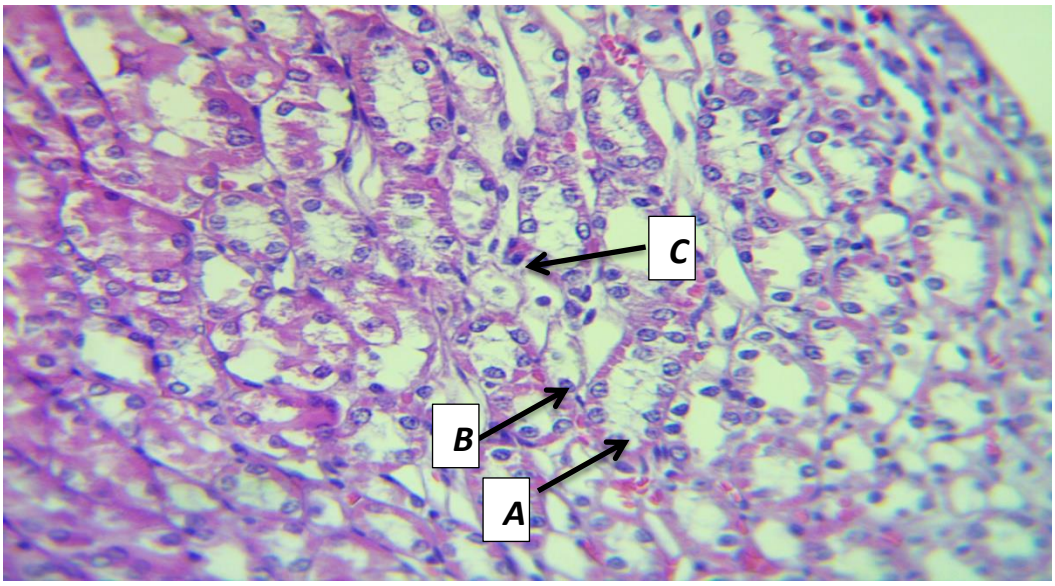

figure (2).Total pulp of the first control group. Renal ducts (A) Fragments of Henle's grafts (B), leukocytes and macrophages interstitial tissue (C). (H&EX40)

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On the other hand, the histological examination of the kidney sections (cortex and pulp) injected with gentamycin indicated degeneration and necrosis of the glomeruli, which belong to the renal granules, and some of them showed dilation, bleeding and atrophy. In the other section, Bowman's area increases, with a sharp expansion of the capsular space, and hemorrhages occur in the epithelial tissue lining the proximal and distant convoluted tubules. Infiltration of inflammatory white blood cells was found in the intertitial tissue between the tubules, as shown in the figure (3)

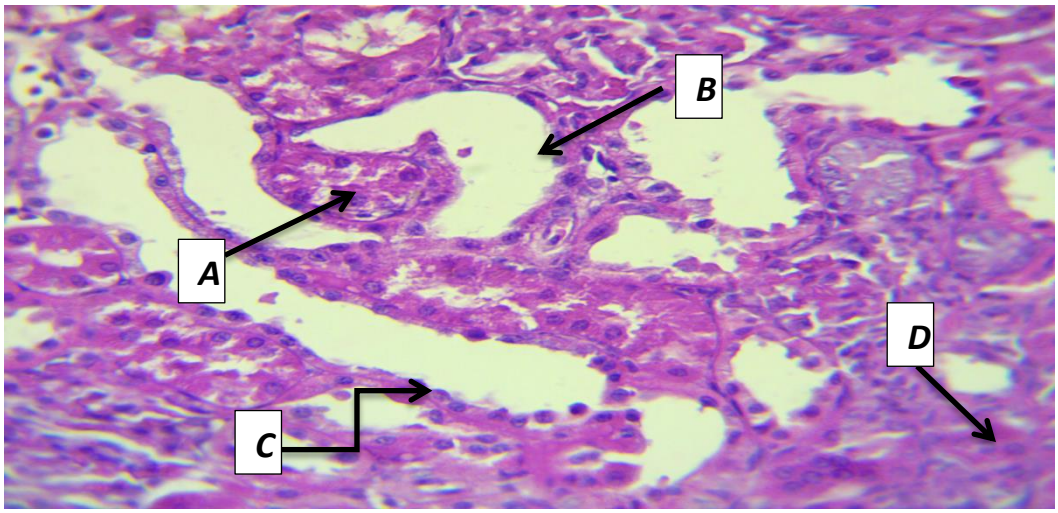

figure (3). which includes gentamycin. Glomerular atrophy (A) Capsular dilatation (B) Dilatation of the lumen of convoluted tubules (C) Interstitial thickening (D). (H&EX40)

While the pulp has a severe expansion of the cavities of the renal tubules and the thin segments of Henle's loop, with the presence of hypertrophy of a number of epithelial cells lining the thin segments of the loop of Henle as in Figure (4).

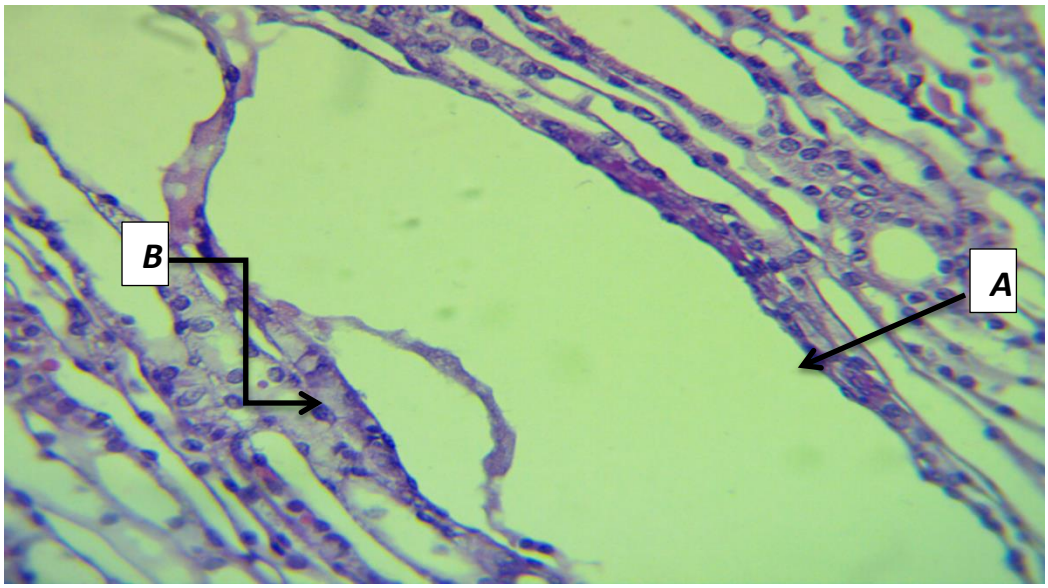

Figure (4). Which includes gentamycin. Severe dilatation of the lumen of the renal tubules and thin segments of the loops of Henle (A) Hyperplasia of the cells lining the loops of Henle (B). (H&EX40)

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Microscopic examination of the gentamycin and verapamil groups indicated the appearance of the renal cortex with mild enlargement with complete rupture of the capsular space around the glomeruli and the presence of thickening of Bowman's capsule with sloughing of epithelial cells in the lumen of the tubules as shown in Figure (5).

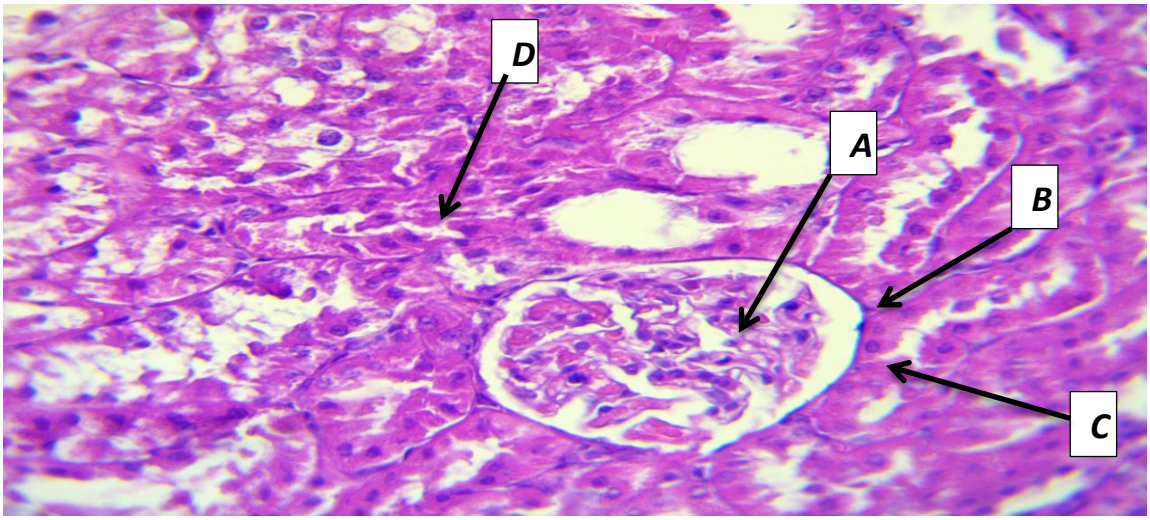

Figure (5). It includes gentamycin and verapamil. renal cortex. Simple glomerulosclerosis (A) Complete rupture of the capsular space (B) Normal Bowman's capsule (C) Epithelial cells in the lumen of the tubules (D). (H&EX40)

As for the medulla of the kidney, most of the cells of the renal tubules show a slight enlargement of the epithelial cells, in addition to the presence of thickening in the basement membrane surrounding the cells lining the tubules, as shown in Figure (6).

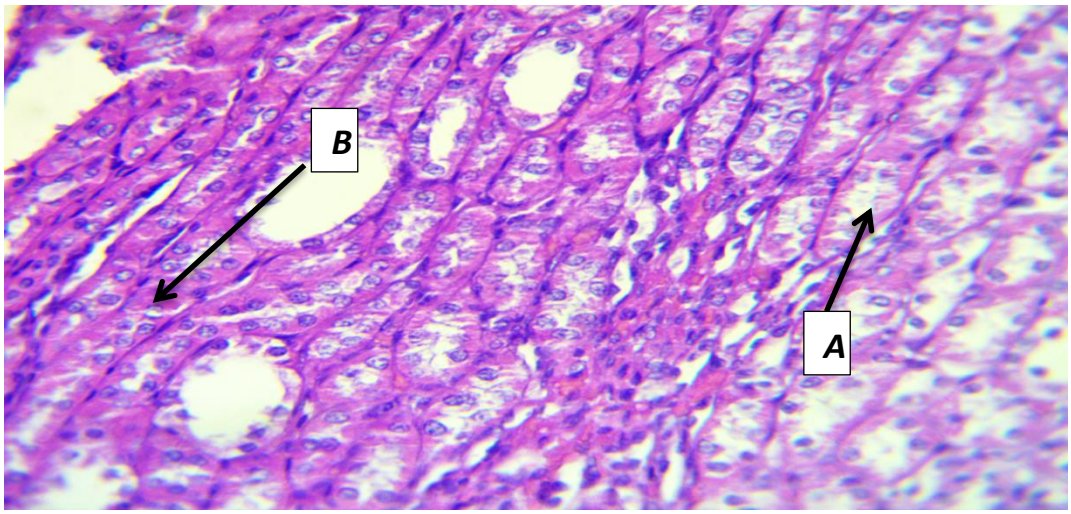

Figure (6). It includes gentamycin and verapamil. College core. With epithelial cells (A), basement membrane (B). (H&EX40)

Discussion:-

Histological examination of renal sections of rats in the gentamicin antibiotic group showed the occurrence of many severe pathological changes, as well as several histological changes,

including rupture of a number of glomerular walls and severe degeneration of a number of glomerular cells. In addition to the many pathological effects of the renal tubules, including severe necrosis of cells in some of them, edema and bleeding between a number of tubes, and enlargement of other tubules, and the expansion of a group of vessels and the occurrence of bleeding within them were recorded. This agreed with many studies(11)(12).

And it agreed with the findings of (13). The results of the current study showed that the renal cortex of mice after administering gentamicin for 30 days showed noticeable destructive lesions in the form of shrinkage of renal corpuscles with the expansion of renal spaces. While we observed complete atrophy in a number of glomeruli and glomerular capillary shrinkage in other glomeruli in mice treated with gentamicin(14).

The pathological changes observed in renal tissues during the current study may be attributed to the increase in pharmacological stimulation to produce different types of reactive oxygen species, including hydrogen peroxide, superoxide anione and hydroxyl radicals, and the subsequent process of All cellular contents, from the cell membrane to the nuclear contents. Several studies have shown that treatment with different antibiotics, including gentamicin increases mitochondrial-mediated hydroxyl generation(15).

As for the process of destroying cellular contents. The breakdown of the renal cytoplasmic membrane after lipid super-oxidation leads to a loss of osmotic balance and a tendency to increase intracellular calcium levels, leading to cell swelling, the first step in a cascade of cellular reflexive changes(16).

With regard to the group treated with gentamycin and verapamil, the results indicated that verapamil has an average efficiency to protect renal tissue against the toxic effects of gentamycin in changes. With(17)(18).

Reference:-

(1) Dhodi, J. B.; Thanekar, D. R.; Mestry, S. N.; Juvekar, A. R. and Linn, C.C.; (2015). Fruit extract ameliorates gentamicin- induced nephrotoxicity in rats via attenuation of oxidative stress .J. Acut. Dis., 135-140.

(2) Raju, S.; Kavimani ,S.; Maheshwara Rao, V.U.; Reddy, K.S.; Kumar,G.V.; (2011) . Floral extract of Tecoma stans:a potent inhibitor of gentamicin-induced nephrotoxicity in vivo. Asian Pac J TropMed; 4: 680-685.

(3) Luft,F.C.(2002).Gentamicin as gene therapy. Clinical implications ,80 (9), 543-544.

(4) Padmini , M .P. ; Kumar , J.V.; (2012). A histological study on gentamicin induced nephrotoxicity in experimental albino rats. IOSR J. Dent. Med. Sci.; 1(1): 14-17.

(5) Moestrup, S. K.; Cui, S.; Vorum, H.; Bregengard, C.; Bjorn, S. E. Norris, K.;Christensen, E.I.; (1995). Evidence that epithelial glycoprotein330/ megalin mediates uptake of polybasic drugs. The Journal of Clinical Investigation, 96 (3):1404-1413.

(6) Abdel Raheem,I .T.;Abdel Ghany, A A. Mohamed G.A.;(2009).Protective effect of quercetin against gentamicin-induced nephrotoxicity in rats. Biol Pharm Bull; 32: 61-7.

(7) Selimoglu,E.;(2007). Aminoglycoside-induced ototoxicity. Current Pharmaceutical Design, 13 (1), 119-126.

(8) Randjelovic, P., Veljkovic, S., Stojiljkovic, N., Sokolovic, D., & Ilic, I. (2017). Gentamicin nephrotoxicity in animals: current knowledge and future perspectives. *EXCLI journal*, *16*, 388.

(9) Drury R, Wallington E.(1980). Carleton's histological technique. 5th ed. New York and Toronto: Oxford University Press; p. 183–184.
(10) Morgan,G.A.;Leech,N.A.;Gloecner,G.W.andBarrett,K.C.(2010).SPSS.for introductory statistic :use and interpretation.2nd ed.Lawrenz Erlbum associatiates,publisers Mahwah, New Jersey London
(11) Ahmadvand,H.;Tavafi,M.;Asadollahi,V.;Jafaripour,L.;Hadipour Moradi,F. ; Mohammadrezaei-Khoramabadi,R.;Khosravi,P.; Salehi, H.;and Cheraghi, A.; (2016). Protective Effect of Carvacrol on Renal in Rats Zahedan J Res Med Sci.; 18(4):e6446
(12) Walaa, F. A., Felemban, E. M., Shafie, A., Alhomrani, M., Habeeballah, H., Alsharif, K., & Farid, M. A. (2019). The Antimicrobial Resistance and Prevalence of Enterococcus Species in Saudi Arabia. *J Pure Appl Microbiol*, *13*(4), 2461-2470.
(13) Mahmoud, A. M., Ahmed, O. M., & Galaly, S. R. (2014). Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats. *EXCLI journal*, *13*, 98.
(14) Veljković, M.; Ilić, S.; Stojiljković, N.; Velicković, L.; Pavlović, D.; Radenković, M.; Branković, S.; Kitić, D . and Ignjatović,G. M. (2015). Beneficial effects of Green tea extract in Gentamicin-induced acute renal failure in rats Sci. J. Faculty of Medicine in Niš;32(1):51-58.
(15) Doroshow,J.H.;and Davies,K.J.;(1986). Redox cycling anthracyclines by cardiac mitochondria.II.Formation of superoxide anion ,hydrogen peroxide, and hydroxyl . radical .J .Biol Chem.,261:3068-3074.
(16)Silan,C.;Uzun,O.;Comunglu,N.U.;Gokcen,S.;andCengiz,M.;(2007).Gentamicn– induced nephrotoxicity in rats ameliorated and healing of effects resveratrol.Biol.Pharm.Bull.,30:79-83.
(17) Akindele, A. J., et al. (2014)."Protective effect of selected calcium channel blockers and prednisolone, a phospholipase-A2 inhibitor, against gentamicin and carbon tetrachloride-induced nephrotoxicity." *Human & experimental toxicology* 33.8: 831-846.
(18) Patil, Amol N., et al. (2014). "Comparison of the species-sensitive effects of different dosages of calcium and verapamil on gentamicin-induced nephrotoxicity in rats and rabbits." *Toxicology International* 21.3: 225.