Comparison of the therapeutic role of garlic extract and verapamil against gentamicin-induced nephrotoxicity in rats

Hmood Sh. Subhi[1], Siham A. Wade[2], Idrees khalaf thamer[3]
[1,2]D Dept. of Pharmacology, Collage of Veterinary Medicine, Tikrit University
[3]prof. of Histology and Anatomy, Collage of Veterinary Medicine, Tikrit University
DOI: 10.47750/pnr.2022.13.S01.40

Abstract

Background: The aminoglycoside group, especially gentamicin, is the critical antibiotic in severe Gram-negative bacterial infections through its significant risk of nephrotoxicity. There are many protective factors for the kidneys, including blocking the flow of calcium to the damaged cells of the kidneys and reducing renal blood flow through the drug verapamil, In the same regard, garlic has been proven to be an antioxidant, a natural plant remedy for kidney protection.

Aim of the study: Knowledge of enzymatic changes in the kidney due to gentamicin treatment and the role of garlic alcohol extract and verapamil in improving these changes.

Methods and materials: 40 rats (3 to 6 months old) were brought up at normal temperature and divided into four equal groups, each group included 10 rats, including the negative control group, which were given orally 0.9% physiological salt solution, and the second group, the positive control (treated with G) 80 mg/kg by intraperitoneal injection, and the third group was given (G) 80 mg/kg intraperitoneally with (V) 3 mg/kg orally, And the fourth group had been injected with (G) 80 mg/kg intraperitoneally with (GAE) 20 mg/kg orally. The study included the evaluation of some biochemical blood parameters, including: levels of creatinine, urea, total protein, sodium, potassium, malondialdehyde (MDA), glutathione (GSH), and catalase (CAT). Above these criteria were the study of the median lethal dose (LD50).

Results: The statistical analysis of the results showed: - The results of the current study, through the results of the statistical analysis, showed a significant increase ($P<0.05$) in the effectiveness of the levels of urea, creatinine, sodium, Dimalone Dehyde (MDA) when comparing the positive control group treated with (G) at a concentration of 80 mg/ In contrast, the levels of total protein, potassium, glutathione (GSH), catalase (CAT) significantly decreased ($P<0.05$) in the positive control group when compared with the other experimental groups.

Conclusion: Garlic alcoholic extract and verapamil are highly effective in protecting blood biochemical parameters from gentamicin-induced nephrotoxicity.

Keywords: Gentamicin nephrotoxicity; Garlic alcohol extract; Verapamil; malondialdehyde; GSH; CAT.

INTRODUCTION

Gentamicin, one of the aminoglycoside antibiotics, is a broad spectrum antibacterial drug that has been playing a role specifically for the treatment of Gram-negative bacterial infections since the beginning of the twentieth century(1) .Although aminoglycosides have many benefits, they have a variety of side effects, the most serious and common of which are ototoxicity and nephrotoxicity caused by antibiotics(2) .Unfortunately, nephrotoxicity is the most notable adverse effect associated with administration of gentamycin as it leads to damage to the tubules and consequently to morphological changes in the histological structure of the kidneys(3). The mechanism of gentamicin-induced cell damage is not fully understood, but the effect of gentamicin in the formation of free radicals, which is the main cause of pathological events, has been reported(4).Moreover, the release of reactive oxygen species is a clear indicator of gentamicin-induced nephrotoxic events, as ROS binds to some large molecules, leading to cell damage and necrosis through multiple mechanisms including protein scavenging and formation of lipid free radicals present in membranes thus degrading DNA(5).The nephrotoxic effects of gentamicin are enhanced by both morphological and functional effects. For example, large renal blood flow and therefore a higher relative exposure to toxic effects, including higher oxygen uptake, sensitivity of renal tissues to hypoxia, and degree of permeability of proximal tubules. As for the presence of verapamil, it is a protective association from toxicity, preventing the flow of calcium to the damaged

cells and reducing the renal blood flow, thus mitigating the development of acute renal failure (6). As for garlic, Allium sativum belongs to the Aillaceae family, and it is considered the second most important green crop, second in sequence after onions, and it is likely that its area of origin is an Asian region It is a green vegetable crop with abundant nutritional and medicinal benefits, as its cloves contain 31% carbohydrates and 2.6% proteins based on wet weight. It is also rich in phosphorous, iron, potassium, magnesium, thiamine, riboflavin, niacin and ascorbic acid (7). Garlic contains the main compound Allicinen, which is characterized as one of the compounds that play a key role in eliminating a large number of viruses, bacteria and fungi (8). Experimental and clinical studies confirm that garlic has beneficial effects and a role in the prevention of various metabolic disorders and diseases and toxicity (9).

Objectives of the study

1- The objective is to compare the protective effect of alcoholic garlic extract and verapamil against gentamicin-induced nephrotoxicity.

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.

-2Preparation of garlic alcohol extract

Weigh 200 g of garlic plant with 1000 ml of 70% alcoholic extract and leave the solution with continuous stirring by the vibrating device for 24 hours at room temperature. Then the solution was filtered through several layers of gauze, then the extract was placed in a dish and left exposed at room temperature to dry, then the extract was collected and placed in a sterile glass bottle and kept in the refrigerator until the experiment was conducted.

-3Preparation 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.

-4Gentamicin 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.

-5Experimental groups and methods

The first experiment :

A- Determination of the median lethal dose of gentamicin by intraperitoneal injection using the up and down method (Dixon, 1980).(10)

The median LD50 of gentamicin was determined by intraperitoneal injection using the up-and-down method. In the experiment, rats aged 3-6 months with weights ranging between (300-200) g were used. The median lethal dose of gentamycin was determined by peritoneal injection based on preliminary experiments in rats. We fixed the dose of gentamicin and then divided the rats into four groups, each group containing four animals. The first group, consisting of 4 rats, was injected with a dose of 674 mg/kg, the second group was injected with a dose of 690 mg/kg, the third group was injected with a dose of 700 mg/kg and the fourth group was injected. At a dose of 710 mg/kg, and after 24 hours, the final result was read (animal survival is symbolized by the symbol O and death is symbolized by the symbol X). The increase and decrease in the dose was by 10 mg/kg and it was found in the first group that the dose did not affect the rats and in the second dose as well, while half of the animals were killed in the third group and in the last dose of the fourth group all animals were killed and the result was compared with the lethal dose measurement table with the use of The law of the method for obtaining the median lethal dose based on the table mentioned before (Dixon, 1980) Up & Down method.

Using the following equation:

LD50=Xf+Kd

The LD50 is the median lethal dose.

Xf: The last dose used in the experiment.

K: tabular value.

d: The constant increase or decrease in the given dose.

B- Determination of the median lethal dose of verapamil by oral administration using the up and down method (Dixon, 1980).

The median LD50 of verapamil was determined by oral administration using the up-and-down method. In the experiment, rats of 3-6 months of age with weights ranging between (300-200) g were used. The median lethal dose of fabramil was determined by oral dosing based on preliminary experiments in rats. We fixed the dose of verapamil and then divided the rats into four groups, each group containing four animals. The first group consisting of 4 rats was injected with a dose of 114 mg / kg, the second group was injected with a dose of 120 mg / kg, the third group was injected with a dose of 125 mg / kg and the fourth group was injected At a dose of 130 mg/kg and after 24 hours, the final result was read (animal survival is symbolized by the symbol O and death is symbolized by the symbol X). The dose increase and decrease were 5 mg/kg. In the first group, it was found that the dose did not affect the rats, and in the second dose as well, while half of the animals were killed in the third group, and in the last dose of the fourth group, all animals were killed. On the table mentioned before (Dixon, 1980) Up & Down method.

Using the following equation:

LD50=Xf+Kd

The LD50 is the median lethal dose.

Xf: The last dose used in the experiment.

K: tabular value.

d: The constant increase or decrease in the given dose.

Second experiment:

It was divided into groups, including:

The first group: included 10 rats, which were given orally a physiological salt solution at a concentration of 0.9% once a day for a month, and this group was considered a negative control group.

The second group: this group included 10 rats injected with the antibiotic (G) at a concentration of 80 mg/kg once daily for a consecutive month inside the peritoneum.

The third group: This group included 10 rats that were dosed with (V) at a concentration of 3 mg/kg orally and injected with the antibiotic (G) at a concentration of 80 mg/kg intraperitoneally once a day for a month.

Fourth group: This group included 10 rats that were dosed with (GAE) at a concentration of 20 mg/kg orally and also injected with the antibiotic (G) intraperitoneally at a concentration of 80 mg/kg once daily for a month.

-6Animal sacrificing and the collection of blood samples

The body weights of all animals included in the current study were recorded exactly 24 hours after the last dose of the experiment in all groups and after the end of the dosing period, the animals were anesthetized with diethyl ether, and the rats were fixed in the cork by pins, then without dissection The heart The blood was withdrawn from the heart directly without dissection by means of a heart puncture, that is, blood is drawn directly from the heart, and the blood volume is transferred (5) ml to a tube without anticoagulant (Gel tube). And the dimensions of all the fatty tissue attached to the organ, as it is placed in a package containing a solution (10)% formalin for the purpose of the histological study of the kidney organ in the study.

-7Preparation of the serum samples

Blood samples were collected by heart stab and placed in a gel tube designated for separating blood components from serum, then centrifuged at (3000) rpm for ten minutes. After the expiration period, the serum was transferred to a test tube (Appendroff tubes) and kept in a freezer for the purpose of estimating standards. The biochemistry of the blood later.

-8Statistical 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 significant.(11)

Results:

A- Determination of the median lethal dose of gentamicin by intraperitoneal injection using the up and down method (Dixon, 1980).

The median lethal dose of gentamycin in rats was determined by using the up-and-down method after injecting different doses of gentamycin into the protein membrane, and the dose value was 700 mg/kg of body weight, as shown in Table (1).

Table No.(1) Determination of the median lethal dose of gentamycin

result	Measurements
The peritoneal LD50 of gentamycin	700 mg\kg
Dosage range	710–674 mg\kg
first dose	674 mg\kg
last dose	710 mg\kg
The amount of ascent and descent in the dose	10 mg\kg
The number of rats per cherub	4 (OXOX)
Tags	Six hours after the injection, signs of stopping movement and abstaining from eating begin to appear, rapid heart rate and difficulty breathing

O:- Symbol for animal survival within 24 hours of injection

X:- A symbol of the animal's death within 24 hours of injection

B- Determination of the median lethal dose of verapamil by oral administration using the up and down method (Dixon, 1980).

The median LD50 of verapamil in rats was determined using the up-and-down method after injecting different doses of verapamil orally, and the dose value was 125 mg/kg body weight, as shown in Table (2).

Table No.(2) Determination of the median lethal dose of verapamil

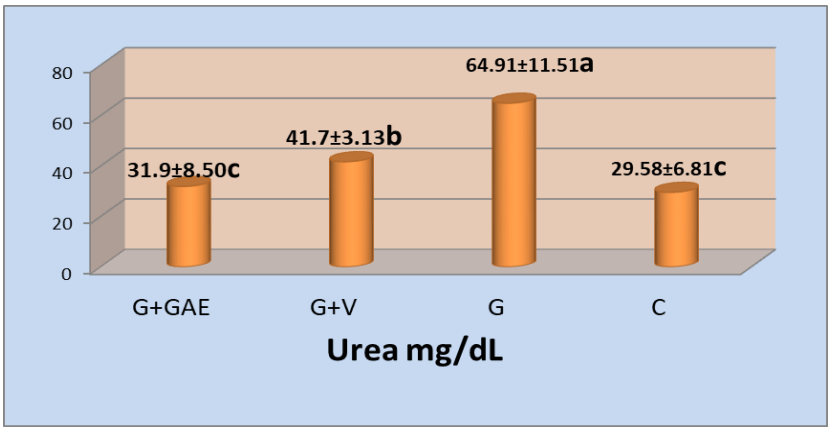
result	Measurements
The median LD50 of verapamil by oral administration	125mg\kg
Dosage range	114–130 mg\kg
first dose	114mg\kg
last dose	130 mg\kg
The amount of ascent and descent in the dose	5mg\kg
The number of rats per cherub	4 (OXOX)
Tags	Hours after the injection, signs of calmness began to appear on the animals, lethargy and abstinence from eating and drinking, with a very clear slowing of the heartbeat.

O:- Symbol for animal survival within 24 hours of injection

X:- A symbol of the animal's death within 24 hours of injection

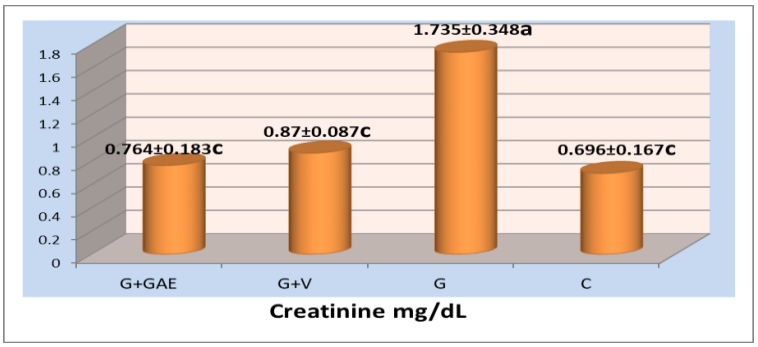
2- Effect of gentamicin, gentamicin with verapamil, gentamicin with garlic on urea concentration.

Treatment with gentamycin showed a significant increase (P<0.05) in urea concentration (64.91 ± 11.51) compared to the control group (29.58 ± 6.81) with the group of gentamycin and verapamil (41.7 ± 3.13) and the group of gentamycin and garlic (31.9 ± 8.50) subject to the study as shown. In Figure (1).



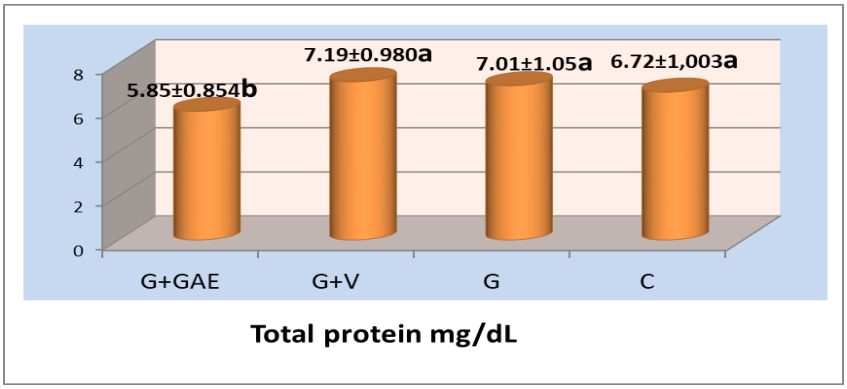
3- Effect of gentamicin, gentamycin with verapamil, gentamicin with garlic on creatinine concentration.

Treatment with gentamycin showed a significant (P<0.05) increase in creatinine concentration (1.735 ± 0.34) compared to the control group (0.696 ± 0.167) with the group of gentamycin and verapamil (0.87 ± 0.087) and the group of gentamicin and garlic (0.764 ± 0.183) under the current study as shown in figure (2).



4- Effect of gentamycin, gentamycin with verapamil, gentamycin with garlic on total protein concentration.

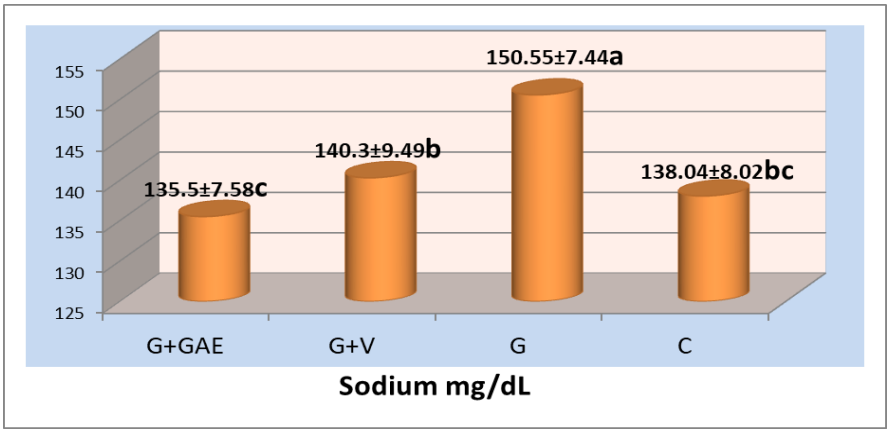
The results of our current study showed that there was no significant increase in the total protein level of the group treated with gentamycin (7.01 ± 1.05) compared to the control group (6.72 ± 1,003) with the group of gentamycin and verapamil (7.19 ± 0.980), while the group of gentamicin and garlic showed a decrease (5.85 ± 0.854) Significantly compared to the control group and the other groups under the current study, as shown in Figure (3).



5- Effect of gentamicin, gentamycin with verapamil, gentamicin with garlic on sodium concentration.

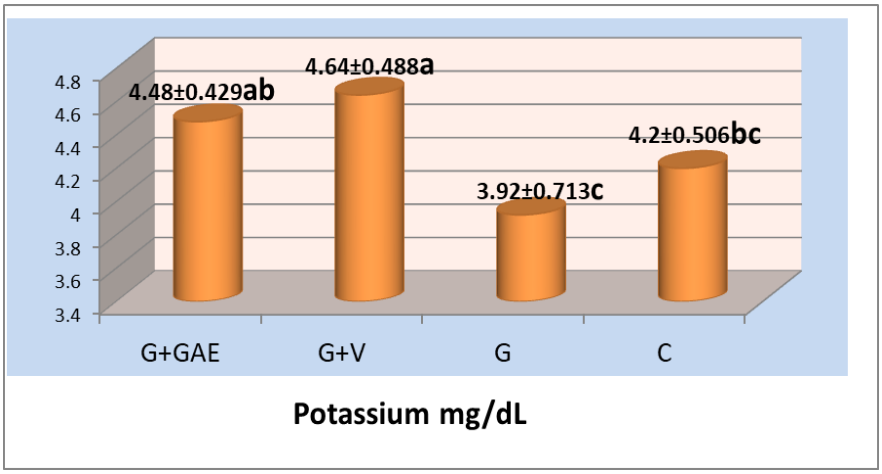
The treatment with gentamycin showed a significant (P<0.05) increase in sodium concentration (150.55 ± 7.44) compared to the control group (138.04 ± 8.02) with the group of gentamycin and verapamil (140.3 ± 9.49) and the group of gentamycin and

garlic (135.5 ± 7.58) under the current study as well. Shown in Figure (4).



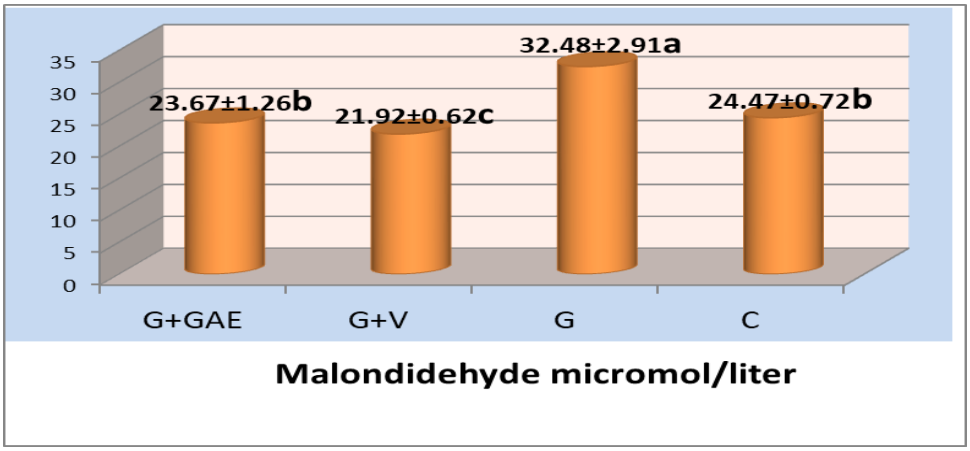
6- Effect of gentamicin, gentamicin with verapamil, gentamicin with garlic on potassium concentration.

The results of our current study showed a significant decrease in potassium level for the group treated with gentamicin (3.92±0.713) compared to the control group (4.2±0.506). While the group of gentamicin and verapamil (4.64±0.488) and the group of gentamicin and garlic (4.48±0.429) showed a significant increase compared to the group treated with gentamicin (4.48±0.429). The control and gentamycin group under the current study is shown in Figure (5).



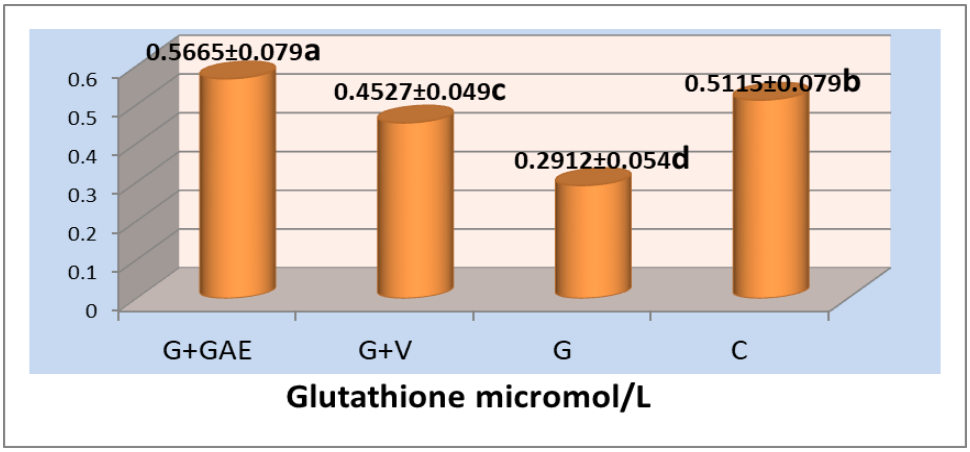
7- Effect of gentamicin, gentamycin with verapamil, gentamicin with garlic on malondialdehyde concentration.

Treatment with gentamycin showed a significant increase (P<0.05) in the concentration of malondialdehyde (32.48±2.91) compared to the control group (24.47±0.72) with the group of gentamycin and verapamil (21.92±0.62) and the group of gentamycin and garlic (23.67±1.26) under study. current as shown in Figure (6).



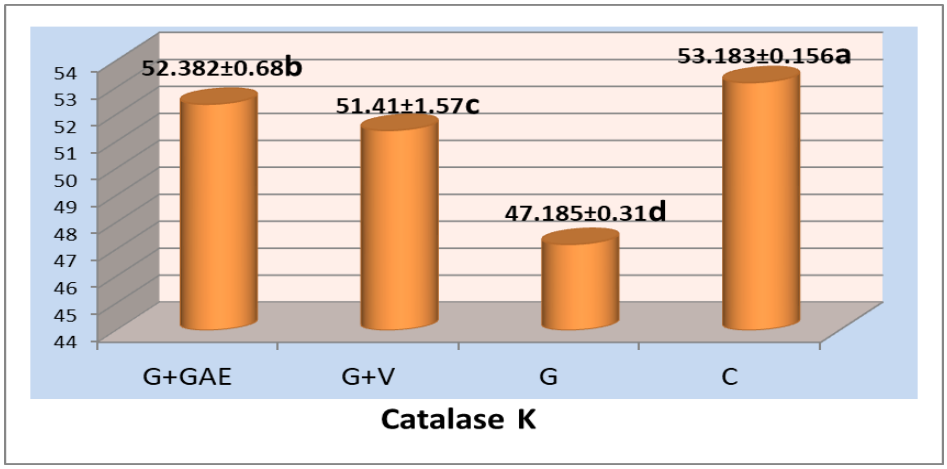
8- Effect of gentamicin, gentamycin with verapamil, gentamicin with garlic on glutathione concentration.

The results of our current study showed a significant decrease in the level of glutathione for the group treated with gentamicin (0.2912 ± 0.054) compared to the control group (0.5115 ± 0.079). While the group of gentamicin and verapamil (0.4527 ± 0.0498) and the group of gentamycin and garlic (0.5665 ± 0.079) showed a significant increase compared to the group. The control and gentamicin group under the current study is shown in Figure (7).



9- Effect of gentamicin, gentamicin with verapamil, gentamicin with garlic on catalase concentration.

The results of our current study showed a significant decrease in the level of catalase for the group treated with gentamicin (47.185 ± 0.31) compared to the control group (53.183 ± 0.156). While the group of gentamicin and verapamil (51.41 ± 1.57) and the group of gentamycin and garlic showed a significant increase (52.382 ± 0.68) compared to the group treated with gentamicin. The control and gentamicin group under the current study is shown in Figure (8).



Discussion

The results of our current study showed that urea and creatinine levels were significantly increased in the gentamicin-treated group compared to the other groups, a result consistent with several studies. (12)(13) It also agreed with (14)(15).

The two confirmed that gentamicin causes a significant increase in the concentration of some biochemical indicators of kidney function, such as blood urea nitrogen (BUN) and creatinine in serum and urine total protein excretion. The interpretation of this result is that gentamicin treatment may cause damage to some glomerular cells, as shown in The current study, leading to a significant increase in urea and creatinine levels. As for the combination of gentamicin with verapamil, there are studies indicating the nephroprotective effect of calcium channel blockers (verapamil) in the nephrotoxicity caused by gentamicin. Possible mechanisms may be blocking calcium influx into damaged cells, vasodilating effects that improve renal blood flow and thus modulating the development of acute renal failure, or antioxidant activity that can reduce the generation of reactive oxygen species(16) The results were in agreement with (17).

As for the results obtained by the group of gentamycin with garlic, they showed the protective role of garlic because it contains

a high percentage of antioxidants, which improves the oxidative stress caused by gentamicin through the events of nephrotoxicity, raising the levels of urea, creatinine and a decrease in total protein(18).

The results in the current study of sodium showed a significant increase in sodium level in the group treated with gentamicin compared to the control group and other groups. This result agreed with some studies(19).

Any abnormal stimulus to which the kidney is exposed activates inflammatory reactions, which leads to an increase in the weight of the kidney, which gives clear evidence of the enlargement of the organ. It is possible that the increase in the weight of the kidney may explain the disruption of the process of water reabsorption, which resulted in dehydration of treated animals as a result of retention Fluids and their accumulation in the blood vessels due to the pathological effects of gentamycin in the walls of the renal glomeruli and what caused the rupture of those walls, as well as the necrosis and severe dissolution of the cells of those glomeruli as prove (20) Regarding potassium, it showed a significant decrease in body weight and an increase in kidney weight(21).

As for the gentamycin group with verapamil, calcium channel blockers (verapamil) were used as a means to reduce calcium entry into the cell in an attempt to reduce the damage related to excess sodium. Verapamil is an effective calcium channel blocker in maintaining renal function, including sodium, in various animals exposed to nephrotoxicity caused by renal ischemia due to gentamicin. Regarding potassium, verapamil increased blood flow in the kidneys. The increase in blood flow (correction of microcirculation) also corrected the morphological changes which reduced the retention of urea and creatinine(16).

While the gentamicin group with garlic, garlic improves kidney function by reducing kidney failure, because it has diuretic properties, as diuretics help to get rid of sodium and water by forcing the kidneys to excrete more sodium in the urine, which reduces the flow of fluids through the vessels The lower the amount of fluid, the less pressure is placed on the walls of the arteries, and thus garlic protects the kidneys from the possibility of harmful effects(22).

The results of the current study showed a significant increase in the concentration of malondialdehyde, in contrast to the significant decrease in the concentration of reduced glutathione and catalase in the group treated with gentamycin compared to the control group and other groups, and the results were in agreement with what was confirmed by many studies(23)(24)(25).

The kidneys are the most affected and damaged organ due to the increased production of reactive oxygen species, which consist of large amounts of long chains of unsaturated fatty acids in the lipid component of the kidneys. The aminoglycoside group is a nephrotoxic antibiotic that causes nephrotoxicity due to increased production of free radicals and decreased antioxidant activity in them(24)(25).

By stimulating the super-oxidation of lipids, gentamicin releases negative hydroxyl radicals and hydrogen peroxide radicals, which bind to some important molecules in cells such as DNA, RNA, proteins, lipids and enzymes, causing oxidative stress, causing many pathological changes. In renal cells, leading to kidney disease, renal hypoxia, and then acute renal failure Perhaps this is what actually happened in the current study, it is also possible that liver tissues are affected by harmful free radicals, and due to reduced levels, the defense system in these tissues loses the ability to clean and process them, reducing glutathione and hydrogen peroxide. Enzymes are produced as a result of their depletion in removing these toxins, providing evidence of hepatotoxicity and nephrotoxicity(23).

The observed decrease in glutathione and catalase levels may be due to the negative effects of antibiotics on the concentrations of reduced glutathione and catalase as in glutathione, which in turn contributes mainly to the conversion of glutathione molecules of the oxidized form to the reduced form(26) It has proven(27)(28).

That gentamicin interferes with the reconstitution of reduced glutathione to the oxidized form by inactivating the enzyme glutathione reductase, which means that the concentration of reduced glutathione was significantly reduced. Reactive oxygen.

In contrast, the high concentration of malondialdehyde led to the ability of gentamicin to form free radicals in the liver and kidneys of rats, which leads to a significant increase in lipid peroxidation products. In contrast, endogenous antioxidants such as glutathione and catalase were reduced, as they destroy the fatty acid-rich network membrane Unsaturated fat increases fat oxidants, which eventually leads to the production of a product called malondidehyde, which in turn damages the membranes of kidney cells, leading to impaired kidney function (29).

Regarding the gentamycin-verapamil group and the gentamicin-verapamil group, there are studies indicating the nephroprotective effect of calcium channel blockers (verapamil) on gentamicin-induced nephrotoxicity. Possible mechanisms may be blocking calcium influx into damaged cells, vasodilating effects that improve renal blood flow and thus modulating the development of acute renal failure, or antioxidant activity that can reduce the generation of reactive oxygen species(16).

With regard to the group treated with gentamycin and garlic, the levels of reduced glutathione, catalase and malondialdehyde did not change significantly compared with the negative control group, and this may explain that garlic works to remove free radicals, many researchers showed the antioxidant property of garlic, which is due to the presence of some components

Chemical including organic sulfur and phenolic compounds It is reported that garlic shows antioxidant capacity, including one of the research that analyzed this using in vivo experiments, where amino acids such as alliin represent one of the most important acids in the components of garlic(30) The effectiveness of the antioxidants present in garlic is due to the presence of high amounts of the biologically active substance allicin, which has the ability to endow hydrogen ions to stabilize free radicals, while the lyn, linase, scudenine and selenium found in garlic also possess antioxidant properties(31) It contains many hydroxyl groups that are necessary for the effective removal of free radicals Therefore, the lyn and poly-linases of the garlic plant increase the level of reduced glutathione and catalase, reduce the oxidative form and inhibit the super-oxidation of cellular lipids (oxidants) represented by Malonedialdehyde(32).

REFERENCES

(1) Shaheen, U.; Manzoor, Z.; Khaliq, T .; Kanwal, A.; Muhammad, F.; Hassan, I. J.; Munawar, S. H. and AL-Haq, M.; (2014).Evaluation of nephroprotective effects of Foeniculum vulgare Mill, Solanum nigrum Linn and their mixture against Gentamicin-induced nephrotoxicity in Albino rabbits. Int. J. Pharm. Sci. Rev. Res., 25(1): 1-9.

(2) Pavle, R.; Slavimir, V.; Nenad, S.; Ljubinka, J.V.; Dusan, S.; Milan,S. and Ivan, I. (2012).Salicylic acid attenuates gentamicin- induced nephrotoxicity in rats, Sci. World .J., 23 :1-6.

(3) McWilliam, Stephen J., et al. "Aminoglycoside-induced nephrotoxicity in children." Pediatric Nephrology 32.11 (2017): 2015-2025.

(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) Parlakpinar, H.; Tasdemir ,S.; Polat, A.; Bay-Karabulut, A.; Vardi, N.; Ucar, M.; Acet, A.; (2005)Protective role of caffeic acid phenethyl ester (cape) on gentamicin- induced acute renal toxicity in rats, Toxicology 2(207) 169–177..

(6)Watson, Alan J., et al. "Calcium channel blockade in experimental amino glycoside nephrotoxicity." The Journal of Clinical Pharmacology 27.8 (1987): 625-627.

(8) Chan, Jackie Yan-Yan, et al. "A review of the cardiovascular benefits and antioxidant properties of allicin." Phytotherapy Research 27.5 (2013): 637-646.

(9) Nasri, Hamid, Mehdi Nematbakhsh, and Mahmoud Rafieian-Kopaei. "Ethanolic extract of garlic for attenuation of gentamicin-induced nephrotoxicity in Wistar rats." 7.5 (2013): 376-382.

(10) Dixon, W. J. (1980). Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol, 20, 441-462.

(11) 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.

(12) Salih, N. A.; (2015).Effect of nettle (Urtica dioica) extract on gentamicin induced nephrotoxicity in male rabbits.Asian Pac J Trop Biomed; 5(9): 756–760

(13) Ullah, N.; Khan, M. A.; Khan, T.; and Ahmad ,W.;(2013).Protective Effect of Cinnamomum tamala Extract on Gentamicin -Induced Nephrotic Damage in Rabbits Tropical Journal of Pharmaceutical Research; 12 (2):215-219.

(14) Udupa, V., & Prakash, V. (2019). Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicology reports, 6, 91-99.

(15) Soliman ,K.M.; Abdul-Hamid,M.;and Othman,AL. ;(2007)Effect of carnosine on gentamicin–induced nephrotoxicity.Med.Sci.Monit.13:73-83.

(16) Stojiljković, N., Veljković, S., Mihailović, D., Stoiljković, M., Radovanović, D., & Ranđelović, P. (2008). The effect of calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. Bosnian journal of basic medical sciences, 8(2), 170.

(17) Gandhi, K. C., Shahani, S. R., Dhar, R., & Gawali, S. S. (2020). Comparative study of Verapamil, Diltiazem, and Amlodipineon on Gentamicin induced nephrotoxicity in rats. Asian Journal of Pharmacy and Pharmacology, 6(3), 210-216.

(18) Nasri, H., Nematbakhsh, M., Ghobadi, S., Ansari, R., Shahinfard, N., & Rafieian-Kopaei, M. (2013). Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. International journal of preventive medicine, 4(3), 316.

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

(20) Jain, D.P.; and Somani, R.S.;(2015).Antioxidant Potential of Hesperidin Protects Gentamicin Induced Nephrotoxicity in Experimental RatsJune Austin Journal of Pharmacology and Therapeutics(23): pp2-15.

(21) Seckiner, I., Bayrak, O., Can, M., Mungan, A. G., & Mungan, N. A. (2014). Garlic supplemented diet attenuates gentamicin nephrotoxicity ın rats International braz j urol, 40, 562-567.

(22) Wile, D. (2012). Diuretics: a review. Annals of clinical biochemistry, 49(5), 419-431.

(23) Dodiya,H.;Jain,M.;Goswami,S.;(2011). Study of urinary Biomarkers for nephrotoxicity in wistar rats. J Pharmacol Toxicol; 6: 571-9.

(24) Osman, I.H.;Tantaway, A.A.; (2013).Antioxidant activity and protective effects of commercial propolis on gentamicin induced nephrotoxicity in rabbits-in vitro study. Turk J Biochem; 38(4):15-409. overview. Antioxidants, 9(7), 619.

(25) Ahmadvand, H., Nouryazdan, N., Nasri, M., Adibhesami, G., & Babaeenezhad, E. (2020). Renoprotective effects of gallic acid against gentamicin nephrotoxicity through amelioration of oxidative stress in rats. Brazilian Archives of Biology and Technology, 63.

(26) Khalid G. Washeel, Entedhar R.Sarhat , Talal H. Jabir(2019).Assessment of Melatonin and Oxidant-Antioxidant Markers in Infertile Men in Thi-Qar Province.Indian Journal of Forensic Medicine & Toxicology. 13(4): 1500 -4

(27) Salim J.Khalaf, adeer Hatem Aljader, Entedhar R. Sarhat(2021). Antidiabetic effect of Aqueous Extract of Medicago Sativa withEnhanced Histopathology of Pancreas in Alloxan Induced DiabeticRats. P J M H S.15(2);:492-496.

(28) Luo, Sha, et al. (2020). "Glutathione is involved in detoxification of peroxide and root nodule symbiosis of Mesorhizobium huakuii." Current microbiology 77.1: 1-10.

(29) Entedhar R. Sarhat, Siham A. Wadi. Mutaz S. Ahmed, Shaima N. Mustafa,Thuraia R. Sarhat(2018).Evaluation of Serum Malondialdehyde, Glutathione peroxidase, Superoxide dismutase, and Catalase levels in Hormonal Contraceptives. Tikrit Medical Journal – June-;Vol(24) No(1) :10-20

(30) Sadrefozalayi, S., Aslanipour, B., Alan, M., & Calan, M. (2018). Determination and comparison of in vitro radical scavenging activity of both garlic oil and aqueous garlic extracts and their in vivo antioxidant effect on schistosomiasis disease in mice. Turkish Journal of Agriculture-Food Science and Technology, 6(7), 820-827.

(31) Mandal, S. K., Das, A., Dey, S., Sahoo, U., Bose, S., Bose, A., ... & Ramadan, M. A. (2019). Bioactivities of Allicin and related organosulfur compounds

from garlic: Overview of the literature since 2010. Egyptian Journal of Chemistry, 62(Special Issue (Part 1) Innovation in Chemistry), 1-11.
(32) Kerio, L.C.; Wachira, F.N.; Wanyoko, J.K.; Rotich, M.K.;(2012). Characterization of anthocyanins in Kenyan teas:Extraction and)Identification. Food Chemistry Microbial pathogens and strategies for combating them: science, .;131:31-38.