The Possible Protective Effect OF Panax Ginseng on Gentamicin- Induced Nephrotoxicity of the Adult Albino Rats: (Morphological and Ultrastructural study)

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Abstract: Background: Aminoglycosides are natural or semi-synthetic antibiotics with a heterocyclic structure formed by two or more amino-sugars linked by glycoside bonds to an aminocyclitol ring. The aminoglycosides group include gentamicin, tobramycin, amikacin, netilmicin, kanamycin, streptomycin and neomycin. These drugs are used primarily to treat infections caused by aerobic gram-negative bacteria. Use of gentamicin is now limited due to its toxic effects, mainly on kidney and vestibular system. Herbal products including ginseng has been reported to possess protective effects against drugs induced nephrotoxicity in experimental animals. **Objective:** The purpose of this study was to find out the effect of gentamicin administration on rat kidney and the possible protective effect of ginseng against gentamicin-induced nephrotoxicity. **Methods:** Sixty male adult albino rats, were divided into 5 groups. Group I (control group) served as control and was given1 ml isotonic saline solution/day intraperitoneally (I.P.) for 10 days; Group II (ginseng group) was given 100 mg/Kg/day of ginseng dissolved in 1 ml of distilled water orally for 10 days; Group III (gentamicin group) was given 100 mg/Kg/day of gentamicin intraperitoneally for 10 days; Group IV was given 100 mg/Kg/day of gentamicin intraperitoneally, simultaneously with 100 mg/Kg/day of ginseng dissolved in 1 ml of distilled water orally for 10 days; Group V was given 100 mg/Kg/day of gentamicin intraperitoneally for 10 days followed by 100 mg/Kg/day of ginseng dissolved in 1 ml of distilled water orally for another 10 days thereafter. At the end of the experiment, blood was drawn from each animal by cardiac puncture for renal function tests. Each animal was then sacrificed and the kidneys were excised and then subdivided into two parts for light and electron microscopic examination. **Results:** In group III, weight of the animals decreased and there was significant increase in mean serum urea and creatinine as compared to the controls (group I). Moderate to severe necrotic and degenerative changes in the glomerulus, proximal convoluted tubules and distal convoluted tubules were seen in this group. When the Ginseng and gentamicin were given together (group IV), a statistically significant improvement in the mean body weight along with improvement in renal function tests, there was significant improvement in the histological picture towards the normal. When ginseng was given after gentamicin there were more improvement in the body weight of animals and renal function tests, with significant improvement in the histological picture towards the normal. **Conclusion:** the results of the current study showed that Ginseng has some protective role against gentamicin induced nephrotoxicity.
[Ashraf A. Mohamed, Alaa S. Al-Sagheer and Mohamed D. Sleem **Anatomy and Anatomy and the Possible Protective Effect OF Panax Ginseng on Gentamicin- Induced Nephrotoxicity of the Adult Albino Rats: (Morphological and Ultrastructural study).** *Nat Sci* 2017;15(4):1-12]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 1. doi:10.7537/marsnsj150417.01.

Keywords: Gentamicin, ginseng, nephroprotective role, nephrotoxicity, serum urea, serum creatinine

1. Introduction

Gentamicin is one of the common amino-glycosides that have been used for the treatment of various bacterial infections. After oral administration, gentamicin is not very effective because it is not absorbed to an appreciable extent from the intestinal tract. The recommended routes of administration of Gentamicin are intravenous, intramuscular, intraperitoneal or topical. Its use is now limited due to its toxic effects, mainly on kidney and vestibular system. Nephrotoxic effects of gentamicin treatment are due to its accumulation in renal cortical tubular epithelial cells[1].

Membranous structures that can be damaged by gentamicin include lysosomes, mitochondria and microsomes. Gentamicin nephrotoxicity accounts 10-15% of all cases of acute renal failure. The cells of the proximal renal tubules have the ability to concentrate gentamicin several folds more than plasma levels[2]. Gentamicin binds to the phospholipids of the cell membrane of the renal tubules and enter inside the cells then it binds to subcellular organelles altering the mitochondrial respiration and small amount may be taken up by lysosomes[3].

Herbal products including ginseng have been reported to possess protective effects against drugs induced nephrotoxicity in experimental animals[4]. The mechanism by which ginseng exerts its activity is presumably through hypothalamus-pituitary-adrenal axis[5]. **Tran et al.** [6] conducted an experimental study on guinea pigs and stated that ginseng may reduce cell damage induced by toxic substances, act to stabilize cell membranes and

protect tissues from damage by inhibiting lipid peroxidation. These effects may be due to the antioxidant nature of ginseng. Blood urea and creatinine are raised significantly in gentamicin induced nephrotoxicity[7].

Treatment with ginseng had been reported to normalize values of raised blood urea and creatinine. However, role of ginseng on nephrotoxic effects of gentamicin have not received proper attention [8]. The current study was, therefore, designed to evaluate the effects of ginseng on gentamicin induced nephrotoxicity by light and electron microscopic examination.

2. Material And Methods

Animals:

This study was an experimental Randomized Control Trial (RCT) conducted at the experimental Research Laboratory of Al-Azhar University, Faculty of medicine for men Cairo on August 2016. sixty adult albino rats (average body weight 250-300 g) housed under the normal conditions in a special clear sided cages at controlled temperature (23± 3°C), humidity (about 60%), 12 h light and 12 h dark and Food & water were available. Unhealthy and dead animals were excluded from the groups. Strict care and cleaning measures were important factors for keeping the animal in a good condition. All rats were randomly divided into five equal groups (i.e. each = 12 rats) as follows:

Group I (control group):

Rats of this group will be injected with 1 ml isotonic saline solution/day intraperitoneally (I.P.) for 10 days.

Group II (Ginseng group):

Rats of this group were given ginseng (100 mg/kg/day) which dissolved in one ml of distilled water orally for 10 days.

Group III (Gentamicin group):

Rats of this group were injected with gentamicin (100 mg/kg/day) intraperitoneally for 10 days.

Group IV (Ginseng + Gentamicin group):

Rats of this group were given ginseng (100 mg/kg/day) orally and simultaneously injected with gentamicin (100 mg/kg/day) intraperitoneally for 10 days.

Group V (Ginseng/Gentamicin group):

Rats of this group were injected with gentamicin (100mg/kg/day) intraperitoneally for 10 days followed by ginseng (100mg/kg/day) which dissolved in one ml of distilled water orally for another 10 days thereafter.

The body weight of each animal was recorded twice weekly. At the end of the experiment rats were anaesthetized with 60mg/kg ketamine (Katalar) then blood samples were collected with cardiac puncture for biochemical investigations like blood urea and creatinine to detect the toxic effects of the drug on the renal function.

Chemicals:

Ginseng: "Ginseng" syrup 120 ml was obtained from "Pharco Pharmaceuticals - Alexandria - Egypt". Each 100 ml of syrup contains ginseng extract 933 mg (9.33 mg/ml). The dose was calculated according to body weight of each rat as 100 mg/kg/day[9].

Gentamicin: "Garamycin" ampoules 40 mg/ ml was obtained from "Memphis Company for Pharmaceutical and Chemical Industries - Cairo - Egypt". Each 1 ml of garamycin ampoule contains 40 mg gentamicin sulphate. A toxic dose of 100 mg/kg/day was calculated according to the body weight of each rat[10].

Kidney specimen collection:

The anaesthetized rats were subjected to midline abdominal incision. The viscera were abducted to one side to expose the posterior abdominal wall. Fine dissection around the abdominal aorta was done to expose it properly Perfusion of the kidneys for washing with saline was done according to**.** A canula was inserted retrograde into the abdominal aorta at a point just proximal to the distal bifurcation of the aorta. Then, ligation of the abdominal aorta was done below the insertion of the canula following by cutting of the inferior vena cava. Physiological saline solution which had been heparinized and warmed to 37°c was perfused retrograde through the aorta. Immediately after onset of perfusion the aorta was clamped just above the origin of the renal arteries. Within 20 seconds the blood was entirely washed out of the kidneys as evidenced by complete blanching of these organs[11]. After sacrificing the animals, The kidneys were excised and then subdivided into two parts for light and electron microscopic examination.

Tissue processing for ordinary histological examination:

Immediately after sacrificing the animals, their kidneys were taken and fixed by immersion in Bouin´s solution for 24 hours**.** The specimens were dehydrated in ascending grades of alcohol, cleared in benzol and embedded in a hard grade paraffin with a melting point between 55°c and 62°c for 3 hours, then the paraffin blocks were prepared[12]**.** Using microtome, sections were cut serially at 5 micron thickness. The sections were spread on glass slide and deparaffinised with xylol. Sections were mounted on glass slides and stained with the following stains:

1- Haematoxylin and Eosin stain H&E[13]**.**

2- Periodic acid-Schiff's reaction PAS[14]

Tissue processing for electron microscopy examination:

Principles:

The wavelength of a voltage-accelerated electron beam is very short; thus it gives it the potential for a much greater resolving power that obtained using light rays. This led to the design of an electron microscope (EM) in which an electron beam is used in place of light rays. Electrons from an electrically heated cathode through the evacuated microscope column. The cathode is a v-shaped tungsten filament. The electrons are accelerated towards the anode which has an aperture through which the electron beam passes before being focused by the condenser lenses onto the specimen. The power of magnification of the electron microscope can be more than X250.000 and its resolving power is better than 0.5nm[15].

Biomarkers of nephrotoxicity (Serological tests):

1. Determination of serum creatinine[16]:

A. Principle: Creatinine is commonly estimated by the alkaline picrate method to form an orange-colored complex with picric acid in alkaline medium. The absorbance of this complex can be measured calorimetrically at 546nm. B**. Calculation:**

Concentration of creatinine in serum Creatinine (mg/dl) =

[illegible]

2. Determination of serum urea:[17]

A. Principle:

Enzymatic determination of serum urea depends on the urease-modified Berthelot reaction. Urease hydrolyzes urea producing ammonia according to the following reaction.

[illegible] $_2$ [illegible] $_3$ [illegible] $_2$

B. Calculation:

Concentration of urea in serum:

Urea (mg/dl)=

[illegible]

3. **Results:**

1-light microscopic findings:

Light microscopic examination of the kidney of the control albino rats and ginseng treated rat revealed a normal corpuscular and tubular histological structure (Figs. 1 & 2). The normal renal corpuscle consisted of glomerulus (capillary tuft) and Bowman's capsule. The glomerulus was a globular network of densely packed anastmosing capillaries. The numerous nuclei in the glomerulus were those of the capillary endothelial cells, mesangial cells and podocytes which can be identified by EM. The Bowman's capsule was formed of the parietal layer characterized by its flat nuclei of the squamous cells lining it, while the visceral layer was closely applied to the glomerular capillaries. The Bowman's space (glomerular space) was the space between the parietal layer and the glomerular tuft (Figs.1&2). The proximal convoluted tubules (PCT) appeared rounded, and were lined by a single layer of short columnar cells with spherical nuclei. The free ends of these cells had well-developed brush borders that almost fill most of the lumen (Figures 1&2). The distal convoluted tubules (DCT) were lined with simple cuboidal epithelial cells with centrally located nuclei. The lumen of distal convoluted tubule was wider with more defined lumen (Figs. 1&2).

In gentamicin treated rats, degenerative changes were markedly apparent within the renal corpuscles, proximal tubules and distal tubules (Figure 3). The renal corpuscles showed dilated capsular space with condensed irregular glomerular capillary tuft probably due to their congestion. Tubular affection was more evident, some proximal tubules can identified however the Tubular affection was more evident, some proximal tubules can identified however the remaining convoluted tubules not identified either proximal or distal due to luminal dilatation with shedding of its lining cells in the dilated lumen with loss of brush border. Also there was decrease in the height of its epithelial cell lining with vacuolated cytoplasm and pyknotic nuclei. Vascular congestion and focal hemorrhagic areas were noticed within the renal cortex as well with massive inflammatory infiltrate (Fig.3).

In the combined gentamicin and ginseng treated rats the renal corpuscle, proximal and distal tubules were nearly similar to the control group except slight presence of vacuolated tubular epithelial lining in some tubules with some congestion (Fig. 4).

When ginseng was given after stoppage of gentamicin there was more evident improvement of the glomerulus, proximal convoluted tubules and distal convoluted tubules which appeared nearly similar to the control group (Fig.5).

2-Electron microscopy findings:

Examination of ultrathin section of the kidney of control and ginseng groups revealed the usual component of glomeruli which is formed of anastmosing capillary loops. The glomerular capillary wall formed of endothelial inner layer, thin glomerular basement membrane and an outer layer of large podocytes (Fig.6). The podocyte has a large nucleus, abundant cytoplasm, thick primary cytoplasmic processes and many secondary foot processes rests on the glomerular basement membrane of the capillary loop. The lumen of the capillary loop may contain RBCs (red blood cells). The glomerular basement membrane is uniform in thickness. Fenestrations were seen between the foot processes (Fig.6). In between the loops the mesangial cells were seen. These mesangial cells were characterized by darkly-stained nuclei surrounded by little electron-dense cytoplasm (Fig.6).

Gentamicin-treated rats revealed dilation and congestion of the glomerular capillary lumen with thickening of the basement membrane. The nucleus of the podocyte appeared irregular with dark nuclear condensation adjacent to the nuclear envelop in addition to the fusion or complete destruction of the secondary foot processes of the podocytes. Multiple electron lucent vacuoles of different sizes, shape were seen within and in-between the podocytes (Fig.7).

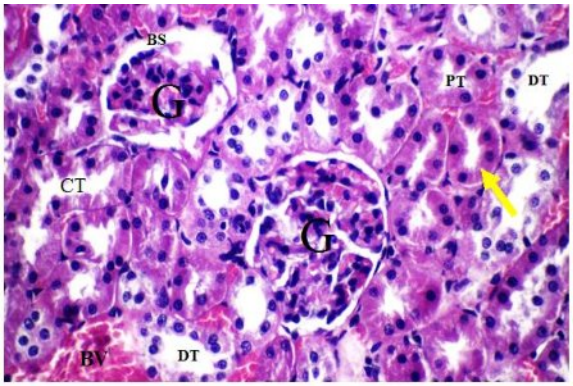

Fig. (1): A photomicrograph of a section in the kidney of the control group, the gilomerulus surrounded by Bowman's space **(BS)..** The proximal tubules showing intact basement membrane and average brush borders **(yellow arrow)**. The peritubular blood vessels **(BV)** in the interstitium are normal. **(H&E. X360)**

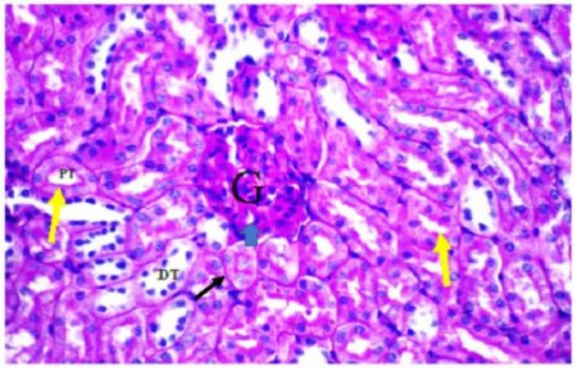

Fig. (2): A photomicrograph of a section in the kidney of the ginseng group showing renal corpuscles formed of glomerulus **(G)** with normal glomerular capillaries **(arrow head).** The proximal convoluted tubules **(PT)** are lined with cuboidal or low columnar cells with spherical central nuclei and acidophilic cytoplasm. The distal convoluted tubules **(DT)** are lined with low cuboidal cells having spherical nuclei and less acidophilic cytoplasm. The renal tubules showing preserved basement membrane **(black arrow)** and brush borders **(yellow arrow). (PAS. X360)**

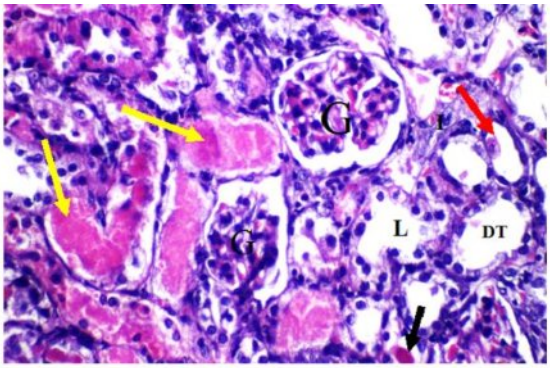

Fig. (3): A photomicrograph of a section in the renal cortex belonging to rat from gentamicin group showing distorted hypercellular glomeruli **(G)**, massive tubular necrosis **(yellow arrows)**, intra-tubular cellular debris **(red arrow)**, intra-tubular casts **(black arrow).** The tubules have edematous wall and dilated lumina. Infiltrating inflammatory cells **(I)** around the tubules can be seen. **(H&E. X360).**

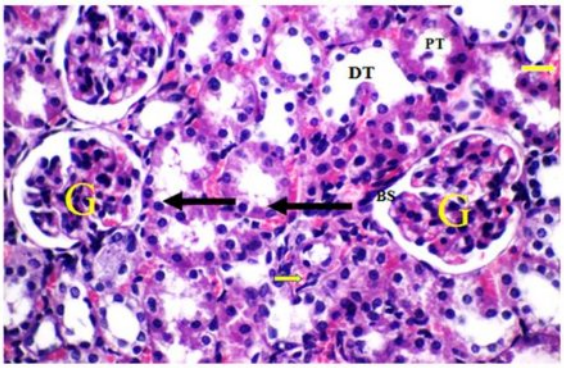

Fig. (4): A photomicrograph of a section in the renal cortex of a rat in group V showing two normal glomeruli **(G)** having normal Bowman's spaces **(BS)** and normal glomerular capillaries. The proximal(PT) and distal tubules**(DT)** are nearly normal having regular walls and clear lumina. Most of tubules are less edematous tubular epithelial lining **(black arrows).** few capillaries were seen with slight dilatation and congestion in some places **(yellow arrows head). (H&E. X360)**

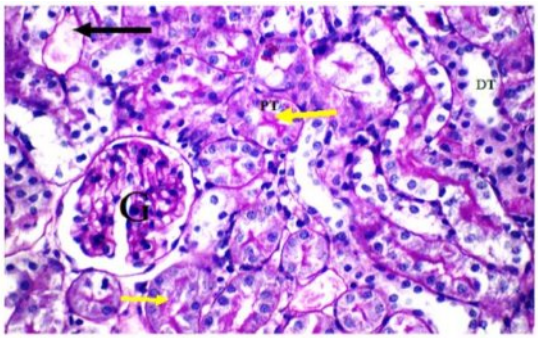

Fig. (5): A photomicrograph of a section in the renal cortex belonging to rat from group IV showing the glomerulus with normal architecture**(G**), most of the proximal convoluted tubules **(PT)** gained brush borders **(yellow arrows)** and others still necrotic **(black arrow)**. Most of the distal convoluted tubules **(DT)** appear normal. No interruption of the tubular basement membrane was shown**. (PAS. X360)**

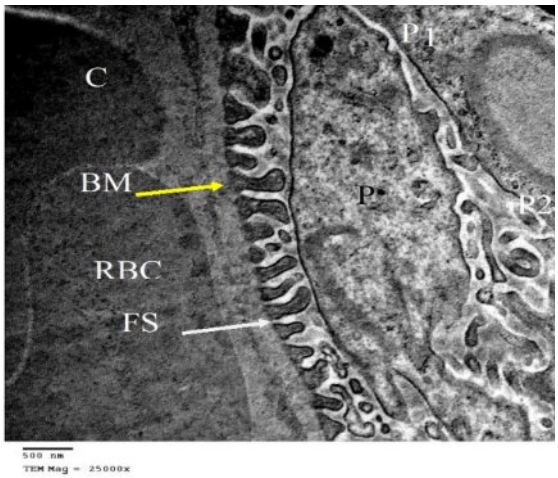

Fig.(6): Electron photomicrograph of highly magnified glomerular capillary of the renal cortex **(C)** of ginseng group containing RBC shows basement membrane **(BM),** fenestration sites **(FS)**, primary foot processes **(P1),** and secondary foot processes **(P2). (TEM. X25000).**

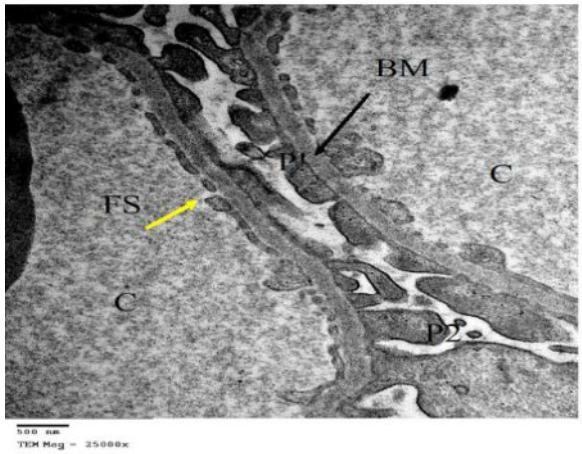

Fig. (7): Transmission electron photomicrograph of highly magnified glomerular capillary **(C)** of the renal cortex of gentamicin group shows loss of glomerular architecture with the processes of podocytes **(P1 & p2)** appear destructed with loss of their uniform appearance. The basement membrane appears thickened with electron dense **(BM)** and widening of the fenestration **(FS). (TEM. X25000)**

In combined ginseng and gentamicin or when ginseng was given after gentamicin (group IV&V**)** showed improvement in the structure of podocytes, regular distribution of the secondary foot processes of podocytes which rests on uniform capillary basement membrane with almost normal capillary lumen were noticed in the renal corpuscle (Fig.8). The ultrastructure of the P.C.T cells in control and ginseng rats appeared with normal structure, having a brush border of numerous microvilli projecting within the tubular lumen and intense cytoplasm due to high content of organelles. The nucleus is spherical, euchromatic, surrounded by numerous mitochondria and apical vacuoles (Fig.9). P.C.T in gentamicin-treated rats showed focal lesions of necrotic tubular cells. The boundaries between epithelial cells of the proximal tubule wall were blurred. Some cells were flattened, with reduced volume, partially, or completely destroyed. The tubular lumen was widened. The brush was focally destroyed. The mitochondria showed abnormal structure. They were edematous with brightened matrix. The cells had only a nucleus and only few organelles, and brightened structure of the cytoplasm. The nucleus of damaged cells was most commonly located in one of the cell poles; its shape was changed and markedly smaller than the nuclei of normal cells. Condensation and peripherally located chromatin were observed (Fig.10).

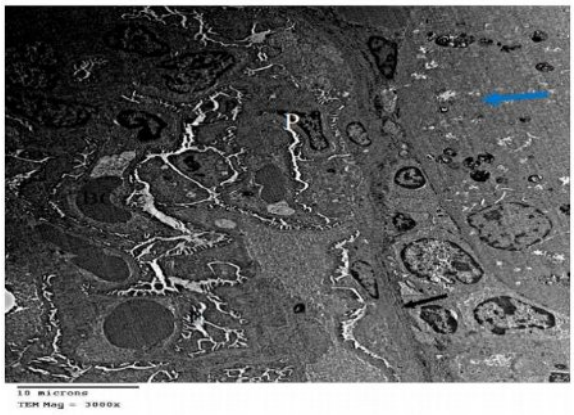

Fig. (8): transmission electron micrograph of the renal cortex of group IV showing thin glomerular basement membrane **(black arrow)** separating the glomerulus from the proximal convoluted tubule **(blue arrow)** and normally appearing processes of podocyte (**P)** but the blood capillaries still congested**(BC). (TEM. X3000)**

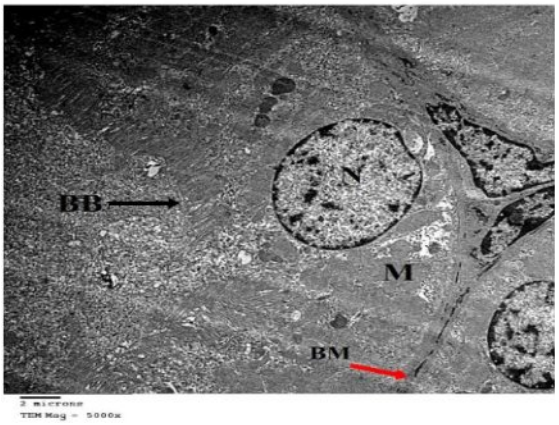

Fig. (9): Electron photomicrograph of part of proximal convoluted tubule of control albino rat. The cell has large spherical nucleus **(N)** and intact basement membrane **(BM).** The cytoplasm containing many mitochondria **(M)**, and numerous microvilli at the luminal side forming clearly obvious brush border **(BB). (TEM. X5000)**

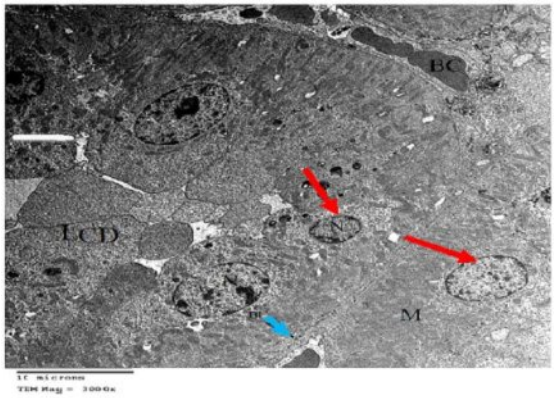

Fig. (10): Transmission electron photomicrograph of the renal cortex of gentamicin group showing a part of the proximal convoluted tubule with multiple epithelial debris inside the lumen **(LCD)** which became obliterated. The apical microvilli are lost **(white arrow).**. Note some nuclei become shrunken with loss of peripheral heterochromatin **(red arrows)** and the basal lamina is interrupted **(BL), (blue arrow)** and there is mitochondria can be seen scattered around the nucleus **(M). (TEM. X3000)**

Fig. (11): transmission electron micrograph of the renal cortex of group V showing part of the proximal convoluted tubules with the nucleus appeared spherical with normal pattern of chromatin distribution **(N).** The brush border appeared normal **(BB).** The mitochondria obviously seen **(M). (TEM. X5000)**

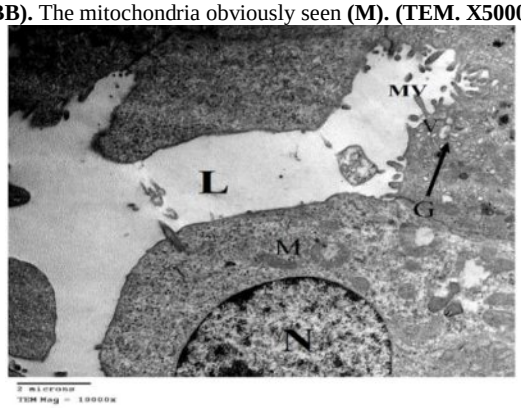

Fig. (12): Electron micrograph of highly magnified distal convoluted tubule cell of the control rats show some microvilli **(MV)**, Golgi apparatus **(G)** and cytoplasmic vesicles **(V)** are in the upper portion of the cell. The mitochondria **(M)** and The nucleus **(N)** are chiefly in the basal region of the cell. **(TEM. X10000).**

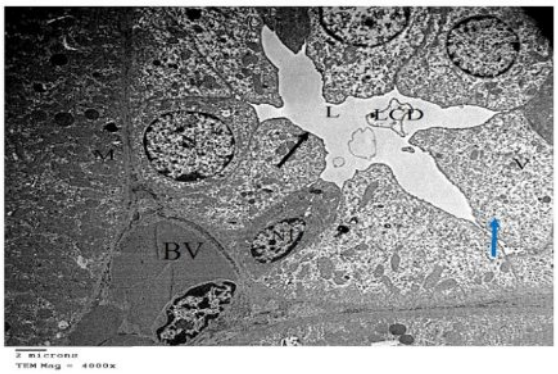

Fig. (13): Transmission electron photomicrograph of the renal cortex of gentamicin group showing part of the distal convoluted tubule with markedly dilated lumen **(L)**, multiple epithelial intra luminal debris **(LCD)** and complete loss of the apical microvilli **(Black arrow)**. Some nuclei appear normal **(N),** the other is shrunken and irregular **(N1)**. Note the cytoplasmic rarefaction and vaculations **(Blue arrow).** The mitochondria are swollen **(M).** The blood vessels are dilated and congested **(BV). (TEM. X4000)**

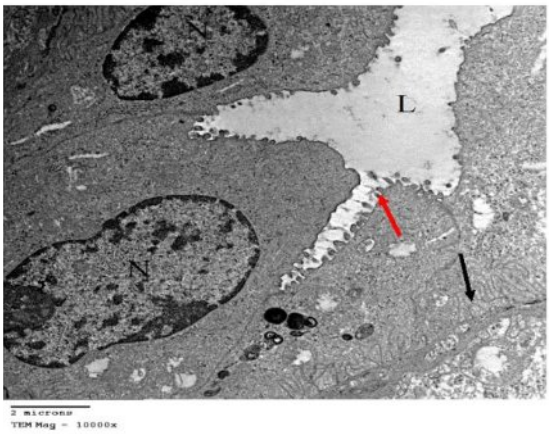

Fig. (14): transmission electron micrograph of the renal cortex of group IV showing part of the distal convoluted tubules with dilated lumen and short microvilli **(red arrow).** The nucleus appeared spherical with normal pattern of chromatin distribution **(N)** and the basal lamina show multiple interdigitating processes **(black arrow). (TEM. X10000)**

Body weight of adult albino rats and serological results:

P.C.T in combined ginseng and gentamicin or when ginseng was given after gentamicin (group IV&V**)** treated rats, the proximal tubular cells revealed marked amelioration compared to those of acitretin group. The nucleus appeared spherical with normal pattern of chromatin distribution, numerous elongated mitochondria, a few apical vacuoles and long microvilli (Fig.11).

D.C.T of the control rats revealed regular basement membrane, sharp luminal outline and wide lumen few microvilli. Moreover, numerous, elongated and round-shaped mitochondria were seen

within their cytoplasm. The cells showed spherical basal nuclei with a central or peripheral electrondense nucleoli (Fig.12). D.C.T in gentamicin -treated rats, the cells was disorganized, disintegrated. The cytoplasmic organelles cannot be identified with irregular shaped nuclei, nucleolus with condensation and peripherally located chromatin with loss of luminal outlines (Fig. 13).

D.C.T cells of combined combined ginseng and gentamicin or when ginseng was given after gentamicin (group IV&V**)** treated rats, showed regular shaped cells with spherical nucleus with central or peripherally located nucleolus with wide lumen with regular luminal out lines (Fig.14). In spite of these signs of improvement cytoplasmic organelles still not identified.

The average of body weight at the start of the experiment were 244, 228.2, 243.6, 230.2 and 246 grams for the five groups of rats respectively. These values were increased gradually in groups I and II. The body weights were decreased after injection of gentamicin in group III with continuous decrease to the eleventh day (the specified day for sacrifice). However, there were an improvement in group IV when gentamicin and ginseng were given together. In group V, body weights were decreased after giving gentamicin alone (in the first 10 days), but it improved later after supplement of ginseng and the animals start to gain weight. These data have been summarized in table (1).

Table (1): The averages of recorded body weight for all groups:

Days(d)	Averages of body weight (g)				
	Group I	Group II	Group III	Group IV	Group V
Day 1	244	228.2	243.6	230.2	246
Day 4	250.3	231.3	239.5	228.2	241.2
Day 7	256.4	236.2	226	228	238.6
Day 11	260.6	239.4	221.2	226.6	237.5
Day 14					238
Day 17					240.2

The sera of blood samples which taken from all rats of group I and II at the end of their experimental period were revealed normal levels of urea and creatinine. However, these two parameters were increased significantly in rats of group III (after injection of gentamicin alone), while they came toward control group in group IV when ginseng were given with gentamicin. In group V there were more improvements of serum urea and creatinine levels. These data have been summarized in table (2).

Table (2): Estimated averages of serum urea and creatinine concentrations of all groups at the end of experimental period for each group.

Groups	Serological parameters	
	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Group I	22.6	0.60
Group II	23.6	0.61
Group III	56.8	2.23
Group IV	35.3	1.30
Group V	31.4	1.26

4. Discussion

Nephrotoxicity is a major complication of the gentamicin (GS) administration. Therefore, the reduction of nephrotoxicity would enhance its clinical value. Several antioxidants that interfere with the production of reactive oxygen species (ROS) have been used successfully to ameliorate GS nephropathy[18].

In the present study, we focused on the effects of Panax ginseng (PG) on the renal damage and oxidative injury induced by gentamicin (GS). In this study, it was shown that treatment with only GS caused nephrotoxicity in rats, evidenced by high plasma urea and creatinine concentrations and by histological lesions (severe tubular necrosis). Similar structural changes were also reported by[19].

The probable mechanism by which gentamicin causes tubular damage is reported to be lipid peroxidation and oxidative injury[20]. Gentamicin enters proximal tubular cells, by interaction between cationic drug and anionic phospholipids of cell membrane as a first step, causing iron release from renal cortical mitochondria and forming iron drug complex, a potent catalyst of free radicals formation[21].

Several dosage schemes have been reported for gentamicin administration. We administered gentamicin at the dose of 100 mg/kg/day interperitoneally which is the dosage scheme reported to cause major nephrotoxicity[22].

Beneficial effects of ginseng are due to the presence of phenolic acids and flavonoids which are responsible for increase in renal blood flow and elimination of free radicals, thus protecting from gentamicin induced oxidative injury. Ginseng protects cell organelles of rats from lipid peroxidation induced by various toxins and cause increase in amount of ribosome in rough endoplasmic reticulum, reflecting its ability to synthesize protein[23].

These findings may induce improvements in renal function. Several antioxidants that scavenge or interfere with production of reactive oxygen species (ROS) have been used successfully to ameliorate gentamicin nephropathy [24]. It is evident from our

work that gentamicin caused tubular necrosis, increase in serum urea and creatinine. However, these effects were mostly ameliorated in the animals simultaneous treated with ginseng indicating that considerable protection is afforded by ginseng however, detailed studies are needed to confirm and study the mechanism of action of ginseng in amelioration of nephropathy.

The level of serum creatinine was considered as one of the most reliable indicator of the efficiency of renal function [25]. The ability of the kidney to filter creatinine was reduced during renal dysfunction as result of diminished glomerular filtration rate (GFR). Thus, the increase in serum creatinine level was an indication of renal [26]. Furthermore, the high level of blood urea is considered significant marker of renal dysfunction [27].

The present work revealed similarity between levels of blood urea and serum creatinine in case of rats belonging to control group and group II that received ginseng only. However, these two parameters were increased in rats that injected by GS alone (i.e. in group III). These results agree with [28].

Our findings revealed that when ginseng was given concomitantly with GS or following stoppage of the latter (in group IV and V respectively), there were decrease of serum urea and creatinine toward the level of the control group. Therefore, ginseng can partially normalize the biochemical parameters when it co-administrated with GS. This in agreement with **Qadir et al., [9].**

Additionally, the results of the present work showed that the use of ginseng led more improvement of these biochemical parameter in case of administration of ginseng after gentamicin (i.e. group V) than that of concomitant administration two drugs (i.e. group IV).

Derakhashanfer et al.,[29] mentioned that ten days of treatment with gentamicin (100mg / kg of body weight) produced remarkable nephrotoxicity characterized by an increase in BUN when compared with the control rats. Vitamin E even in pre-treated or concurrent usage with gentamicin failed to significantly hold the BUN with normal base line. The results of the present work revealed that concurrent use of ginseng gave great protective effects against gentamicin nephrotoxicity more than using vitamin E that is one of the powerful antioxidants.

As regarding to the physical examination, the body weight of the rats belonging to the treated group (gentamicin group) was markedly decreased in comparison with the rats of the control and ginseng groups. However, The body weight isn't significantly affected in groups IV and group V.

Histopathological evaluation of the kidneys was commonly focused on the tubular and interstitial histological changes due to nephrotoxicity effects of aminoglycosides[30]. Some authors have also paid attention to glomerular histological changes [31].

It has been observed in the present study by the light and electron microscopic examination that the renal cortex of the second group of animals that received ginseng (100 mg / kg of body weight for 10 days) revealed normal histological structure with no pathological changes. These results are in agreement with those of **Morsy**[32], which reported that no pathological changes could be observed in the kidney given with either vitamin C or ginseng.

The results of the present work showed that injection of gentamicin caused marked tubular, glomerular and interstitial alterations in the renal cortex of the kidneys of rats (group III). This is generally in agreement with **Alarifi et al., [33]** who found that exposure to gentamicin was capable of inducing marked renal histological harmful alterations in the form of cortical glomerular and tubular alterations.

Lopez-Novoa et al.,[34] reported that tubular damage in GS administration was more prominent in proximal convoluted tubules than distal tubules. They explained that could be due to the fact that proximal convoluted tubules are the primary sites of reabsorption and active transport, thus leading to a higher concentration of GS in the epithelial lining of these tubules.

However, our findings by the use of electron microscopic examination revealed marked alteration of both proximal and distal convoluted tubules in GS administrations. These results are relatively in harmony with the data obtained by **Alarifi et al.,[33]** who found that the tubular alterations affect most of the proximal convoluted tubules and to a lesser extent the distal tubules.

The results of the present work are in agreement with that of Fujiwara **et al., [35]** who proved that the possibility of GS nephrotoxicity was not restricted to the proximal convoluted tubules but extends to the distal convoluted tubules. They recognized that by their findings which showed a swollen, necrotic like cells of the distal tubules caused by GS nephrotoxicity.

In the present results, when GS administrated alone, the proximal and distal convoluted tubules demonstrated edema of the lining cells, cytoplasmic vacuolations, cellular desquamation and loss of brush border of the proximal convoluted tubules. There were marked dilatation of the tubular lumina that showed intraluminal cellular debris or dropped out cells as a result of desquamation and necrosis of the epithelial lining. Interruption of the basement

membrane were also seen. These findings are in agreement with the results of **Souza et al.,**[36] and **Alarifi et al.,**[33] who recorded similar results. They mentioned that GS affect the renal tubules which showed swelling, cytolysis and loss of brush border. Their lumina were filled with degenerated, desquamated and apoptotic cells.

The results of the present work are in agreement with that of **Qadir et al.,**[9] who documented that the injection of gentamicin led to dilatation of the proximal convoluted tubules in the cortex with appearance of patchy necrosis, loss of brush border, presence of cellular debris and accumulation of inflammatory exudates within their lumen. The epithelial cells of the proximal convoluted tubules showed hydropic changes with cytoplasmic vacuolations. Some of the tubules exhibited desquamated epithelial cells in their lumina and the nuclei of these cells were swollen and karyolitic.

Electron microscopic examination revealed that the mitochondria of the tubular epithelial cells of the proximal or distal convoluted tubules appeared rounded swollen and lost their normal elongated appearance.

In accordance with this study, **Ekberge et al.,** [37] stated that the treatment with gentamicin resulted in alteration of mitochondrial shape and inhibition of its function.

The results of the present work showed that the tubular changes by GS caused toxic effects on the distal tubules. This in agreement with autoradigraphic studies by **Silverblatt and kuhen**[38] who demonstrated that transport of amino-glycosides occur in the distal tubules as well as in the proximal tubules. **Parsons et al.,** [39] reported that acute gentamicin- induced hypercalciuria was mediated by a decrease in calcium reabsorption which occur in the early distal tubules.

In the present work, electron microscopic study revealed that the injection of GS caused mesangial hypercellularity, poorly defined glomerular capillaries and changes of Bowman's spaces which varied from narrowing to complete obliteration. these results interpreted by the previous findings reported by **Alarifi et al.,** [33]. This explained the results of the present work which revealed that the effect of GS alone not only limited on the tubular alterations but also it affected the glomeruli.

It was also been reported that gentamicin could induce mesangial cell apoptosis in renal glomeruli rather than some intracellular changes seem to play also a major role in the contractile, proliferative and apoptotic effects induced by GS in renal glomeruli and cultured mesangial cells [40].

Mesangial cells are perivascular pericytes located at the core of the glomerular tuft between capillary loops. Most authors reported that mesangial cell contraction plays a major role in reduction of GFR and glomerular filtration surface which decrease renal function [41&42] **Martinez-salgando et al.,** [40] indicated that in vivo treatment of rats with GS induced a simultaneous increase in glomerular (mainly mesangial) cell proliferation and apoptosis without apparent changes in the number of glomerular cells. According to **Tamura et al.,**[43] both glomerular proliferation and apoptosis have been shown in several glomerulosclerotic lesions. The given dose of GS in the present work led to glomerular cell proliferation, which was marked than apoptosis and thus a net increase in cell number was observed.

Veljkovic et al., [44] documented that subcutaneous injection of gentamicin (100 mg /kg / day for eight days) produced glomerular congestion, narrowing of Bowman's space, periglomerular inflammation and hyaline casts.

As regards the interstitial changes of the present work, the kidneys of gentamicin-injected rats showed cortical interstitial edema with dilatation and congestion of peritubular blood capillaries. the intertubular spaces became widely separated from one another due to the accumulation of edematous fluid. This in agreement with the findings of **Alarifi et al.,**[33].

The findings of the present work also demonstrated that the use of GS alone caused focal areas of massive interstitial hemorrhage with some infiltrating inflammatory cells in these areas and also around the tubules, glomeruli and blood vessels. These finding were in agreement with results of **Alarifi et al.,** [33] **and Osman and tantawy** [45].

It is evident from the present work that GS caused tubular necrosis and increase in serum urea and creatinine. However, these effects were mostly ameliorated in rats received GS and ginseng simultaneously (i.e. group IV). These findings denoting that the toxic changes of GS on the glomeruli, tubules and interstitium were reduced by the antioxidant effect of ginseng. The cells of the proximal tubules had normal nuclei with no intraluminal cellular debris. The tubules appeared less edematous, had highly slightly dilated lumina, less desquamated epithelium with a few dropped out cells. These results are in agreement with that of **Derakhashanfar et al.,** [29] **and Qadir et al.,** [9].

The results of the present work revealed that co-administration of ginseng and gentamicin reversed most of the destructing effect on the glomeruli. Ginseng was reversed the mesangial hypercellularity, decreased the narrowing and obliteration of Bowman's space and improve the capillary loops. This in agreement with **Veljkovic et al.,**[44] who

demonstrated that co-administration of green tea (antioxidant that resemble ginseng) with gentamicin reversed most of the histopathological alteration induced by GS, as the green tea administration alleviated the tubular degeneration of gentamicin, there were no necrosis of epithelial cells of the proximal tubules, and the glomeruli were seen normal.

The present results are in agreement with those of **Qadir et al., [9]** who reported that even with simultaneous use of ginseng and gentamicin, there were focal areas of interstitial hemorrhage, vascular congestion and less inflammatory cellular infiltration. In addition, we added that the intertubular spaces were normal compared to group III that received gentamicin alone.

Rang et al., [46] reported that the GS nephrotoxicity is reversible and renal function recovers if the use of the drug is stopped. So, we can clarify that the detected reversibility of gentamicin-induced structural changes by the use of ginseng after stoppage of GS injection in (group V) not only due to antioxidant effect of ginseng but also due to stoppage of toxic effect of GS which is greatly reversible.

Unique findings has been found in group V where there were complete reversal of the toxic effects of the GS on the proximal and distal convoluted tubules achieved after giving ginseng. This improvement seems to presumably be due to amelioration of GS induced oxidative injury to the tubular system. However, toxic effects of GS on the interstitium of the cortex were not completely reversed. A slight dilatation and congestion of the peritubular capillaries with some mononuclear infiltrative inflammatory cells were still present.

The present results which obtained in group V revealed that the interstitial improvement was less than the tubular improvement but there was no interstitial hemorrhage detected which means more improvement than that of group IV.

Histopathological analysis of the kidneys of the group of rats, which received ginseng orally and GS at the same time showed the mildest changes in comparison to the other groups. The protective effect of ginseng was even more pronounced comparing to the GS injected group. The biochemical parameters and cortical structural and ultrastructural changes of the rats received ginseng after stroppage of GS injection were similar to group I and II.

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