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# Possible Protective Role of Saffron Against Gentamicin-Induced Renal Toxicity in Adult Male Albino Rats: A Histological and Immunohistochemical and Biochemical Study

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## Abstract

*Background:* One of the most prescribed classes of antibiotics is the aminoglycoside family. Gentamicin (Ge) is considered the best frequent member of this family in clinical practice. Although its serum level is within the therapeutic range, its nephrotoxicity; the most prominent adverse effect; might still happen. Furthermost, studies are seeking supplements that could fight against the antibiotics' adverse inflammatory and oxidative stress. Saffron (Sa) has been shown to be an effective antioxidant and anti-inflammatory supplement.

*Aim of Study:* The current research highlights the possible protective role of saffron against gentamicin-induced nephrotoxicity in albino rats.

*Material and Methods:* Forty male albino rats were divided into four equal groups. Group I (Control group) received daily intraperitoneal (IP) injection of 0.3ml distilled water. Group II received Sa extract IP injection daily in a dose of 80mg/kg. Group III received Ge daily intermuscular (IM) injection in a dose of 70mg/kg. Group IV received both Ge and Sa extract in the does as previously described. After 7 days of all group's treatment, the nephrotoxicity was assessed by measuring serum creatinine and urea. Moreover, the tissue antioxidant levels of Glutathione Peroxidase (GPx) and superoxide dismutase (SOD) and genetic expression of Tumor necrosis factor (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ɪcB) were evaluated. Distinguishing the extent of renal tissue structural damage was done by both histopathological and immunohistochemical examination. After conducting the quantitative morphometric evaluations, the results were statistically analyzed.

*Results:* Rats given Ge revealed nephrotoxicity represented by anincrease in blood urea and creatinine as well as acute tubular necrosis in the form of tissue severe congestion, vacuolation, desquamation, and interstitial mononuclear cell infiltration. Collagen fibers were strongly deposited. Caspase-3 immunoreactivity was highly positive. Whereas rats given both Sa and Ge exhibited a decrease in serum urea and creatinine levels as well as a relatively minor degree of tissue necrosis. The biochemical results of both control and Sa-only treatedgroups were the same.

*Conclusion:* Sa extractameliorates the nephrotoxicity induced by Ge antibiotics.

***Key Words:*** *Saffron Extract – Nephrotoxicity – Gentamicin.*

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## Introduction

**THE** kidney is a primary organ responsible for a variety of xenobiotic toxicants, including potentially dangerous environmental chemicals. Extremely high renal blood flow, an abundance of metabolizing enzymes and transporters, and the kidney's capacity to concentrate various solutes during the generation of urine are all characteristics that contribute to the organ's high sensitivity to xenobiotics [1]. Because of their high bactericidal potency, low level of resistance, and positive interactions with beta-lactam antibiotics, aminoglycoside antibiotics are among the most frequently used classes of antibiotics. Gentamicin (Ge) and tobramycin are the members that are used in the clinical field frequently [2]. Ge is bactericidal and effective against Gram-negative and limited Gram-positive organisms. Ge is not metabolized but is distributed essentially unchanged within the extracellular space before excretion in the kidneys by glomerular filtration [3].

Routine use of Ge at a dose of 80mg/kg/body weight is the major and common cause of renal toxicity associated with aminoglycosides in approximately 25% of patients [4]. This toxicity is caused by one or more widespread pathogenic pathways, including thrombotic microangiopathy, glomerular hemodynamic changes, inflammation, crystal nephropathy, and tubular cell toxicity. Destruction of kidney cells was eventually caused by an excess generation of reactive oxygen species (ROS), which damage DNA, proteins, and lipids

within cells [5]. Reduced glutathione (GSH) and superoxide dismutase (SOD) levels have been found to be low in the kidneys upon treatment with Ge. Oxidative stress played an important role in the nephrotoxicity of Ge [6]. Numerous researchers have attempted to reduce the nephrotoxicity caused by Ge and a variety of methods have been used [7]. Treatment of rats with a variety of antioxidants significantly reduces renal dysfunction and tissue damage [8]. Ge-nephrotoxicity has been reduced using herbal extracts. Garlic, Rheum emodi, Aervalanata, and corn silk have all been used to reduce the nephrotoxicity of Ge [9]. Crocus sativus L., generally known as saffron (Sa), is a plant that is commonly used as an antispasmodic, stomachic, expectorant, diaphoretic, gingival sedative, and carminative in folk medicine [10]. Crocin, a component of Sa extract, has a protective impact on oxidative stress carried by ischemia-reperfusion in rat kidneys. Related to its potential to scavenge ROS and enhance antioxidant responses, Sa is known for a variety of functions due to its antioxidant effects [11]. Sa extract did not have a sufficient impact on nephrotoxicity brought on by Ge. So, in this article, we evaluate the potential impact of Sa aqueous extract on Ge-induced nephrotoxicity.

## Material and Methods

*Animals and housing conditions:*

The experiment was involved according to all ethical rules concerning animal research and was approved by the Institutional Animal Care and Use Committee of New Giza University during November 2022. Forty male albino rats weighing between 150 and 200gm were used in the experiment provided by New Giza University. The rats were provided with ordinary rat chow and were housed in wire mesh cages (4 rats/cage) at a controlled temperature (24±1°C). The animals received unlimited access to food and water.

*Chemicals:*

Gentamicin (Ge)vial of 80mg I.M manufactured by Memphis Co. for pharm. & chem. Ind. Cairo-A.R.E. [12].

Preparation of aqueous Saffron (Sa) extract: Crocus sativus L. stigmas was supplied by the Saharkhiz Saffron Co. in the form of a bottle of 110 gram. The stigma was dried in the air and then grounded to a fine powder. The aqueous extract was prepared by adding one gram of saffron stigma powder to 100ml boiling distilled water, after 2 hours it was homogenized, filtered, and stored in dark bottles immediately until use [13].

*Experimental design:*

*Animal groups:*

They were randomly allocated into two categories: Control rats (10 rats) and experimental rats (30 rats). Group I (control): Animal of this group received a daily IP injection of 0.3ml distilled water for 7 days. Group II: Sa group (n=10); received IP injection of Sa extract in a dose of 80mg/kg/day [10] (for 7 days. Group III: Ge group (n=10); received IM injection of Ge in a dose of 70mg/kg/day [12,14] for 7 days. Group IV: Ge + Sa group (n=10); received Sa extract in a dose of 80mg/kg/day IP as well as Ge in a dose of 70 mg/kg/day IM for 7 days.

*Blood biochemical study:*

At the end of the experiment, the rats were anesthetized with diethyl ether and blood samples from the retro-orbital vein were obtained from each animal and were subjected to sera analysis of the following: Serum creatinine and urea.

*Scarification and tissue sampling:*

Following the blood samples extraction, the animals were sacrificed after giving sodium thiopentone IP in a dose of 50mg/kg, the kidney specimens were dissected, and cleaned with distilled water. The right kidneys of all different groups were homogenized in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4). The antioxidant markers were estimated by measuring the renal tissue quantitative activities of Glutathione Peroxidase (GPx) and superoxide dismutase (SOD). Also, the homogenates were used to isolate the total RNA. The left kidneys were fixed overnight in 10% neutral buffered Formalin at 4ºC and embedded intoparaffin blocks for further histopathological and immunohistochemical studies.

*Histomorphometry study:*

Unstained 4µm thicknesses sections were prepared from the former paraffin blocks were allocated for light microscopic examination and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT). Also, they have stained with caspase 3 antibodies.

The quantitative study was performed with an Image J analysis computer software system. The specimen preparations of the kidney from each rat were subjected to quantitative studies in 10 non-overlapping microscopic fields randomly picked from each slide. They were examined within the standard measuring frame of a known area equal to 11694.91 $\mu m^2$. The following parameters were measured: (I) The glomerular basement membrane,

the area of glomeruli, and tubular epithelial height in H&E-stained sections. (II) Area percentage of collagen fibers in MT-stained sections. (III) Area percent of caspase 3 immune-reactivity in caspase 3 immunostained-sections.

*Real-time quantitative PCR of the investigated genes:*

Using the Easy pure RNA Kit, total RNA was extracted fromright kidney homogenates (transgenic, China.catalog no. ER101-01). The Beckman dual spectrophotometer was used to determine the quantity and quality (USA). For highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube in a 48-well plate utilizing the Step One equipment, Transcript Green One-Step qRT-PCR Supermix-Kit, catalogue no. AQ211 had been developed (Applied Biosystem, USA). By using the mean critical threshold (CT) values of the housekeeping gene expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), each target gene was normalized for variation in expression. The examined genes' primer base sequences are as follows: TNF-: (F) 5'-AAATGGGCTCCCTCTATCAGTTC-3', (R) 5'-TGCTTGGTGGTTTGCTACGAC-3'. NF-B: (F) 5' GATCATCAACATGAGAAACGATCTGT-3', (R) 5'TAGCGGTCCAGAAGACTCAG-3'.

*Statistical analysis:*

Statistical analysis was performed using statistical package for the social sciences (SPSS) version 21.0 (IBM Corporation, Somers, NY, USA) statistical software. The data were expressed as means ± standard deviation (SD). Statistical evaluation was done using a one-way analysis of variance (ANOVA). Significance was considered when the $p$-value was equal to or less than 0.05 throughout the study.

## Results

*Histopathological Results:*

*Hematoxylin and Eosin-Stained (H & E) Sections:*

The sections of the control group I showed normal histological architecture of the renal tissue, consisting of renal cortex containing renal corpuscles, proximal convoluted, distal convoluted, and collecting tubules. The renal Malpighian corpuscle was composed of a glomerulus formed of numerous blood capillary loops surrounded by a Bowman' scapsule (Fig. 1-A,B). The sections of Sa group II exhibited normal structure in renal corpuscles and tubules as the control group (Fig. 1-C,D). The sections obtained from Ge group III showed the lining epithelium with vacuolation, and proximal convoluted tubules showed dilatation of their lumens with the fragmentation of their lining epithelium with exfoliated and sloughed epithelial cells (tubular necrosis). Areas of congestion were noted at glomerular capillaries and in between convoluted tubules (Fig. 2-E,F). Sections obtained from Ge + Sa group IV elaborated that most of the proximal and distal convoluted tubules appeared relatively with minimal fragmentation, and vacuolation of their lining epithelium (Fig. 2-G,H).

*Masson's trichrome (MT) stained sections:*

The renal sections of control group I revealed the normal distribution of collagen fibers between the renal tubules and around blood vessels (Fig. 3I). Renal sections of Sa group II showed more or less normal distribution of collagen fibersas a group I (Fig. 3J). Renal sections of Ge group III showed a strong deposition of collagen fibers in between renal tubules and around the blood vessels (Fig. 3K). Sections of Ge + Sa group IV showed mild collagen fiber deposition between renal tubules (Fig. 3L).

*Caspase 3 immunostaining:*

Renal sections of control group I revealed few nuclear immuno reactivities of caspase 3 in the renal tubules (Fig. 4M). Renal sections of Sa group II showed more or less normal immuno-expression of Caspase-3 (Fig. 4N). Renal sections of Ge group III showeda marked increase in Caspase-3 nuclear immunostaining of the renal tubules (Fig. 4O). Sections of Ge + Sa group IV showed mild Caspase-3 immunostaining of the renal tubules (Fig. 4P).

*Quantitative histomorphometric study:*

The Glomerular basement membrane thickness (µm): The thickness of the basement membrane in Ge Group III was significantly ($p$0. 05) increased compared to the control group. There is no significant difference between control Group I&Sa Group II. Although in Ge + Sa group IV, there was a significant ($p$0. 05) thickness decrease comparable to Ge Group III (Table 1, Fig. 5A).

The Mean of the glomerular area in Ge Group III was significant ($p$ 0.05) decrease comparably to the other groups. Although in Ge + Sa group IV, there was a significant ($p$0. 05) increase comparably to Ge Group III (Table 1, Fig. 5B).

The proximal tubular epithelial height in Ge Group III was significant ($p$ 0. 05) decrease comparably to the other groups. Although in Ge + Sa group IV, there was a significant ($p$ 0. 05) height increase comparably to Ge Group III (Table 1, Fig. 5C).

*Mean area% of MT staining:*

Morphometric and statistical studies showed that in Ge group III, the area percent of collagen fibers was significantly larger than that in both control group I and Sa group II. There was no statistically significant difference between control group I and Ge + Sa group IV. While there was a significant decrease in the mean area% of Ge + Sa group IV compared with Ge group III (Table 1, Fig. 5D).

*Themean number of caspase3 immune-reactivity:*

The number of Caspase-3 immuno positive cells was significantly higher in Ge group III compared to the control group I and Sa group II, according to morphometric and statistical findings. In Comparing betweenthe Ge group III and Ge + Sa group IV, there was a significant decrease in the Caspase-3 immuno positive cells (Table 1, Fig. 5E).

*Biochemical study:*

*Blood biochemical study:*

The serum Ureaand creatinine levels in the Ge group III were significantly higher than in the control group I, according to a blood biochemical analysis. Sa showed significantly decreased levels of serum Urea and creatinine in the Ge + Sa group IV (Table 2, Fig. 6-A,B).

*Renal tissue levels of GPX and SOD antioxidants:*

The tissue levels of GPX and SOD antioxidants in Ge group III were significantly decreased than in thecontrol group I, according to a renal tissue biochemical analysis. Sa showed a significant increase in the levels of antioxidants in the Ge + Sa group IV (Table 2, Fig. 6-C,D).

*NF-κB, TNF-α gene expression:*

According to NF-κB gene expression, there is a significant increase in Ge group III as compared to control group I. There was a significant decrease in NF-κB gene expression in Sa received group II and in Ge + Sa received group IV as compared to Ge group III (Table 2, Fig. 6E).

According to TNF-α gene expression, there is a statistically significant increase in Ge group III as compared to control group I. There was a significant decrease in TNF-α gene expression in Sa received group II and in Ge + Sa received group IV as compared to Ge group III (Table 2, Fig. 6E).

Table (1): Quantitative histomorphometric study of all groups.

| Variable | Control group I | Sa group II | Ge group III | Ge + Sa group IV |
|---|---|---|---|---|
| Glomerular basement membrane thickness (μm) | 1.0720±.34006 | 1.0550±.67571 | 2.9560±.54708* | 1.1190±.29422*# |
| Glomerular Area | 227.1110±28.94108 | 220.1430±27.94111 | 127.6370±39.08865* | 206.3330±27.33987*# |
| Proximal tubular epithelial height | 10.7100±1.56216 | 10.2820± 1.78584 | 4.1430± 1.26658 * | 9.5820± 1.79884*# |
| Mean area % of collagen fibers | 9.44±3.08 | 9.01±3.02 | 21.22±5.67* | 12.85±2.81*# |
| Mean number of Caspase-3 immuno positive cells | 4.0±1.0 | 4.3±2.0 | 36.50±6.11 * | 12.10±2.72*# |

Data were expressed as Mean ± SD, *p*-value 0.05 was significant.
(*) Denotes significant as compared to control group I.
(#) Denotes significant as compared to Ge Group III.

Table (2): The biochemical parameters of all groups.

| Variable | Control group I | Sa group II | Ge group III | Ge + Sa group IV |
|---|---|---|---|---|
| Urea (mg/dL) | 18.91 ± 1.21 | 15.81±1.41 | 113.27±17.68* | 48.16±5.92*# |
| Creatinine (mg/dL) | 0.46±0.03 | 0.49±0.02 | 2.36±0.49* | 0.87±0.05*# |
| Mean of GPx tissue level (unit/mg) | 139.3±12 | 135.5±9.4 | 85.9± 11.6* | 145.8± 14.3 *# |
| Mean of SOD tissue level (unit/mg) | 2.35±0.4 | 2.21±0.5 | 0.55±0.2* | 2.89±0.5*# |
| NF-κB | 0.87±0.02 | 1±0.02 | 3.5±0.56* | 2.1±0.13*# |
| TNF-α | 1±0.007 | 1±0.045 | 6.2±0.25 * | 2.5±0.16*# |

Data were expressed as Mean ± SD, *p*-value 0.05 was significant.
(*) Denotes significant as compared to control group I.
(#) Denotes significant as compared to Ge Group III.

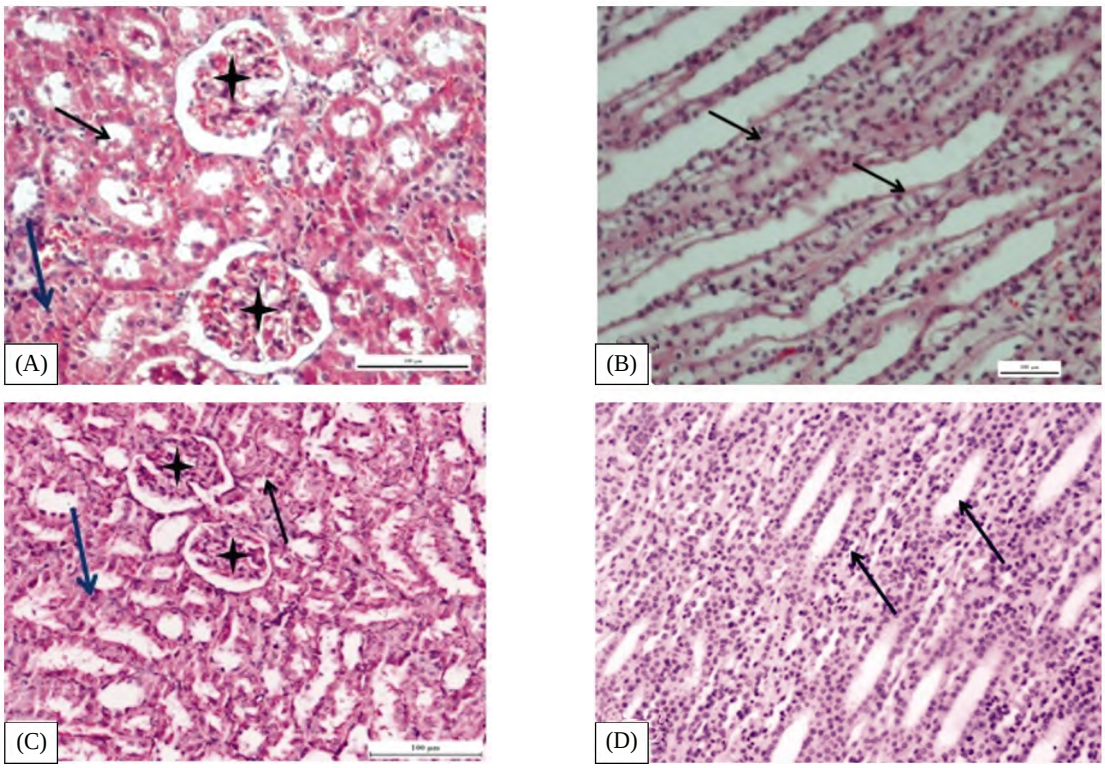


Fig. (1): H & E-stained sections, (A): A section of control group I showing Malpighian corpuscle (stars) with glomerular capillaries surrounded by capsular space and parietal layer of Bowman, s capsule, proximal convoluted tubules (black arrow) and distal convoluted tubules (blue arrow). (B): A section in the renal medulla of the control group showing medullary tubules (black arrows). (C&D): Sections of Sa group II showing nearly normal appearance of the renal cortex (C) and medulla (D) as the control group.

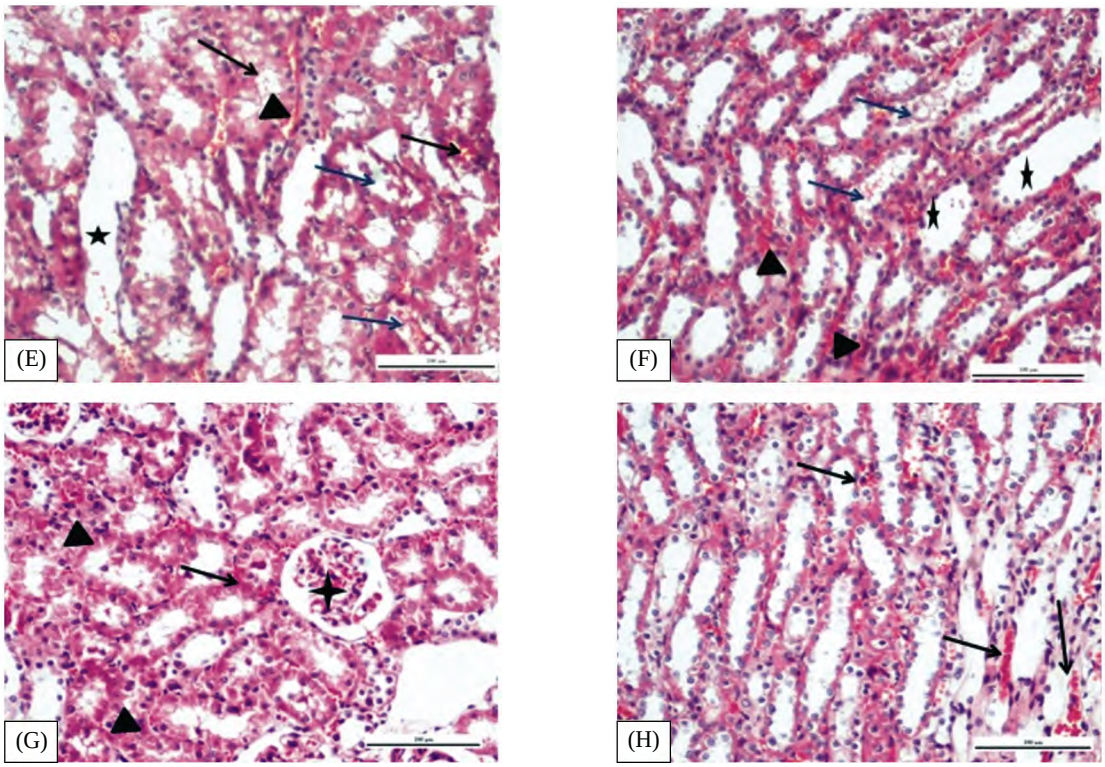


Fig. (2): H & E-stained sections, (E&F): Sections of Ge group III showing Malpighian corpuscle with rupture of glomerular capillaries, escape of RBCs, disappearance of capsular space (black arrows). Cystic dilatation of the tubules (stars), shed cells in their lumen (blue arrows) and interstitial extravasation of RBCs with inflammatory infiltrate (arrow heads). (G&H): Sections of Ge + Sa group IV showingnearly intact renal corpuscles (star) withminimal congestion of blood capillary (arrows) and most of proximal convoluted tubules appeared more or less normal. However, some of them showed fragmentation, vacuolation of their lining epithelium with exfoliated cells in their lumina (arrows head).

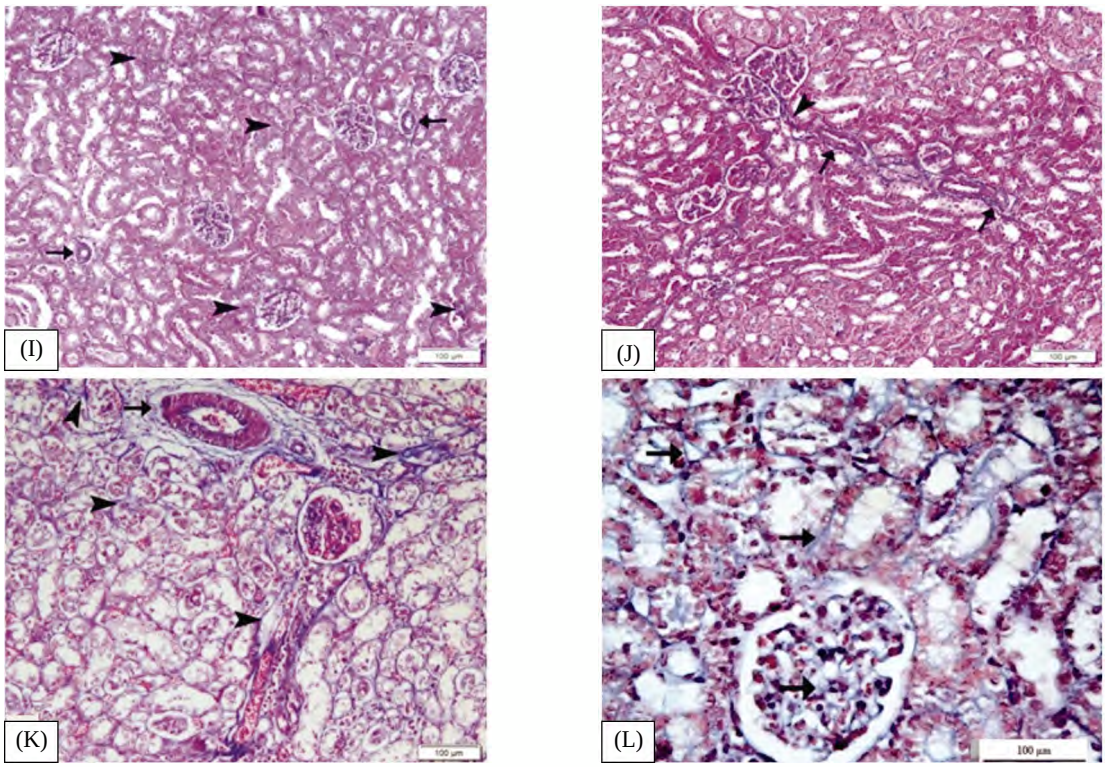


Fig. (3): MT-stained sections, (I): A section of control group I showing normal distribution of collagen fibers between the renal tubules (arrow heads) and minimal amount around blood vessels (arrows). (J): A section of Sa group II showing minimal collagen fibers between the renal tubules (arrowhead) and around blood vessels (arrows). (K): A section of Ge group III showing strong deposition of collagen fibers in between renal tubules (arrow heads) and around the blood vessels (arrow). (L): A section of Ge + Sa group IV showing mild collagen fiber deposition in the renal corpuscle & between renal tubules (arrows).

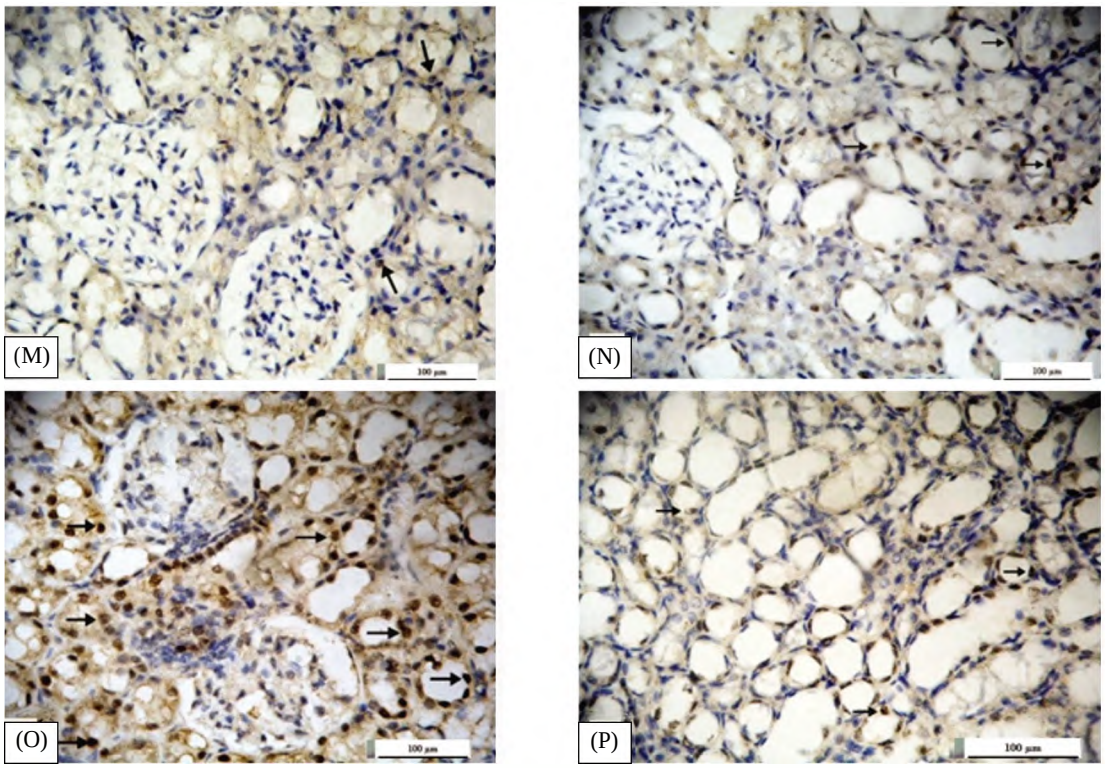


Fig. (4): Caspase-3-stained sections. (M): A section of control group I showing few nuclear immunoreactivity in the renal tubules (arrows). (N): Normal immuno-expression of Caspase-3 in Sa group II as the control group. (O): Showing marked increase in Caspase-3 nuclear immunostaining in Ge group III (arrows). (P): A section of Ge + Sa group IV showing mild Caspase-3 immunostaining in some tubular cells (arrows).

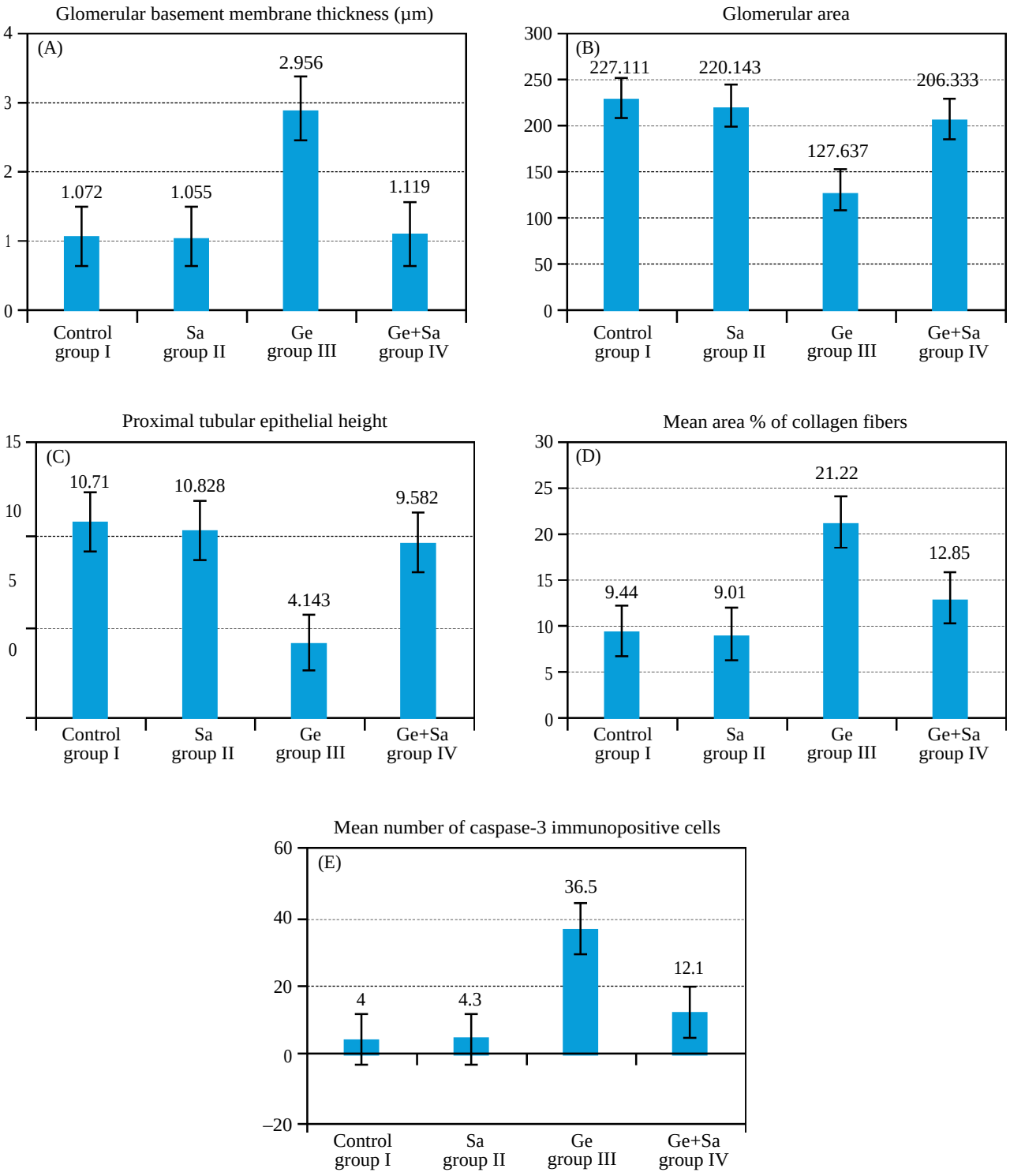


Fig. (5): Comparison between different groups. (A): Glomerular basement membrane thickness (B): Glomerular area. (C): Proximal tubular epithelial height. (D): Mean area % of collagen fibers. (E): Mean number of caspase-3 immuno positive cells.

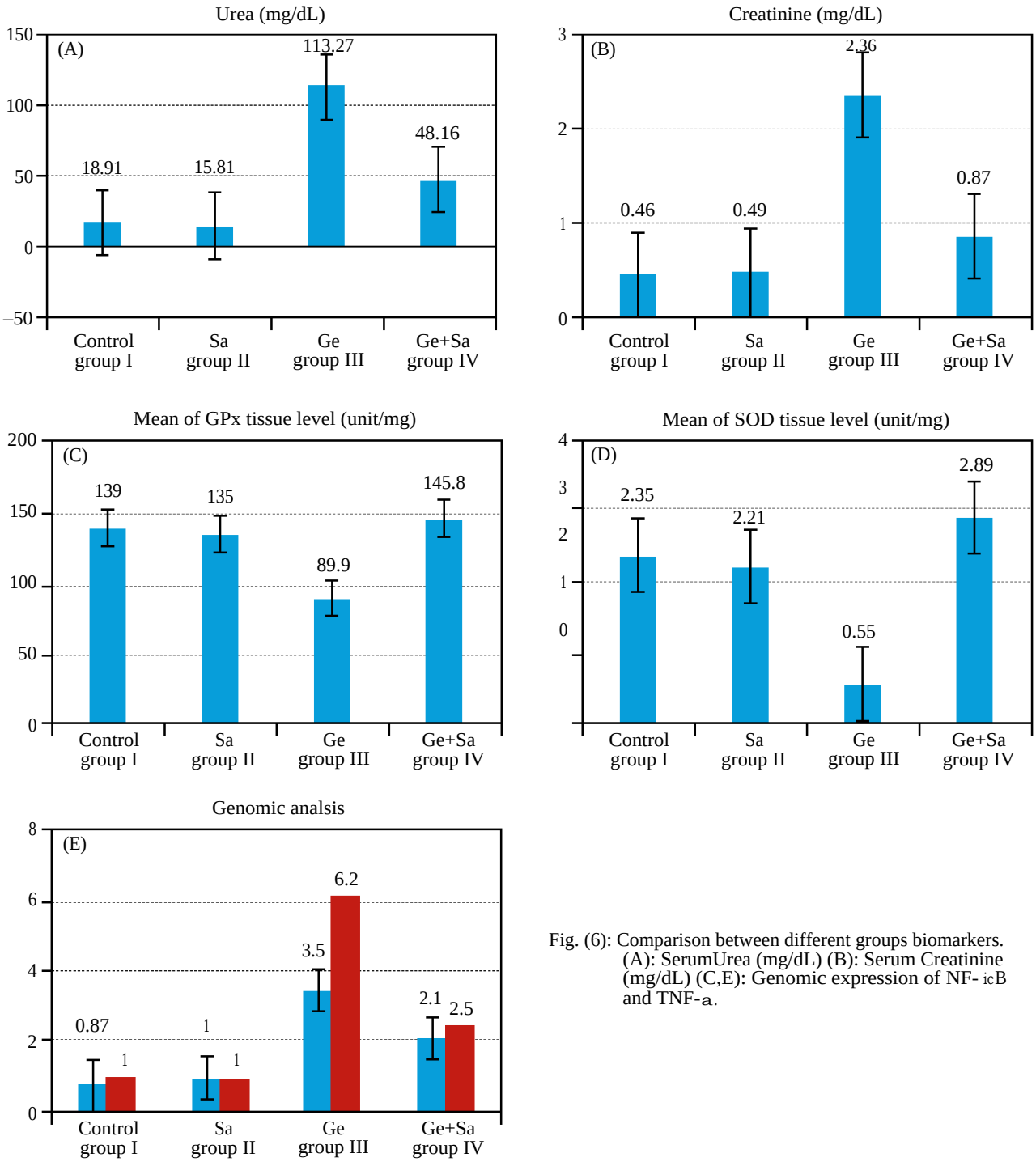


Fig. (6): Comparison between different groups biomarkers. (A): SerumUrea (mg/dL) (B): Serum Creatinine (mg/dL) (C,E): Genomic expression of NF- icB and TNF-a.

## Discussion

Ge has been long suggested by therapeutic guidelines for the treatment of serious infections because it is particularly effective in treating bacteria that are resistant to other antimicrobials. Gram-positive organisms can hardly benefit from its bactericidal and efficient action against the Gram-negative ones **[3]**. This is because mostof Gram-negative organisms seen in communities and hospitals are susceptible to it despite its relatively low levels of resistance and quick bactericidal activity. These characteristics make it a highly beneficial empirical medication when quick management of a serious infection is necessary. However, Ge induces nephrotoxicitywhich is more frequently reversible but ototoxicityis documented less frequently andless typically reversible **[15]**.

The use of Ge is still very frequent in clinical practice despite its nephrotoxicity in (15%-20%) of therapeutic courses. Although the exact mecha-

nism causing Ge nephrotoxicity is unknown, multiple studies have suggested that oxidative stress may be the primary cause of nephrotoxicity. Some researchers claimed that using various antioxidants could reduce Ge-induced nephrotoxicity **[16]**.

This study was designed to evaluate the histopathological, immunohistochemical, and biochemical changes associated with Ge and the protective role of Sa as one of the antioxidants and inflammatory supplements. These results indicated that the Sa extract can reduce oxidative stress, inflammation, functional disturbances as well as tissue damage caused by Ge.

This experimental model revealed histological changes detected mainly in the proximal and to some extent in the distal tubules. The obtained results also proved a strong correlation between histological and morphometric alterations in Ge-induced nephrotoxicity.

In group llI "Ge treated" group, concerning renal corpuscles, there was glomerular distortion with a widening of capsular spaces. Proximal convolute tubules were the markedly affected part, its epithelial lining showed detachment of brush border, dilatation in the lumen with vacuolation, and fragmentation of their cytoplasm. These results in agreement with Amin et al., & Chetankumar Acharya et al., **[15,13]** reported the same finding, which may attribute to the oxidative stress caused by Ge.

In Ge-treated rats, the glomerular basement membrane was significantly thickened. Morphometric analyses of this group compared to the control group I & IV, showed a statistically significant decrease in the proximal tubules' epithelial height, indicating damage to tubular epithelial cells. While morphometric analysis of the glomerular area showed a statistically significant decrease in Ge group III compared to the group I & IV. These outcomes corroborated with Stojiljkovic et al., **[17]** results.

MT-stained sections were employed in the current study to detect fibrosis. In the Ge-treated group, there was statistically significant more collagen fibers deposition within glomeruli and between renal tubules. These findings were in accordance with Aldahmash et al., **[18]**. Renal interstitial fibrosis is brought on by a variety of inflammatory cells and growth factors, such as transforming growth factor-beta 1 (TGF-1), which is largely produced by macrophages. The induction of local myofibroblasts, which produce extracellular matrices like collagen and fibronectin, depends on it Bledsoe et al., **[19]** and Sadek et al., **[20]**. The conversion of interstitial cells, which results in the deposition of collagen, aids in the growth of myofibroblasts as a result of the usage of Ge, which elevates the levels of TNF-a, Farris et al., **[21]**.

Regarding group IV MT-stained sections, there is a significant reduction in the deposition of collagen fibers in the interstitium. This is because Sa suppresses proinflammatory signaling and downregulates proinflammatory cytokines produced by T cells, such as IL-6, IL-2, and TNF-a Hosseinzadeh, **[22]**.

These results demonstrated statistically significant increased immunohistochemistry expression of caspase-3 in the renal cortex of Ge-treated rats, in accordance with Suh et al., **[23]** who reported a significant increase in the expression of the caspase-3 protein in the kidneys treated with Ge. It was reported that Ge-induced apoptosis was characterized by caspase-3 activation Oliver and Vallette, **[24]**. According to Casanova et al., **[25]**, oxidative stress is a key contributor to the onset of kidney damage.

The current work showed that Geinduces oxidative stress and decreases the antioxidant activities in the kidney tissue, which agrees with Ansari et al., **[26]** and Moreira Galdino et al., **[27]**. It has been reported that Geis released into the cytoplasm by lysosomes destruction and damages mitochondria, inhibits electron transport, and impairs ATP production. These alterations result in ROS such as hydroxyl radical, superoxide anion, and hydrogen peroxide Mahmoud, et al., **[28]**. These oxidants also damage cellular proteins and nucleic acids and lead to membrane lipids peroxidation Katary and Salahuddin, **[29]**.

This study showed the antiapoptotic properties of aqueous Sa extract because it significantly reduced the expression of caspase-3 in group IV compared to group III treated only with Ge. This was confirmed statistically. According to previous research, the nephrotoxicity of Ge was brought on by the generation of free radicals, an increase in intracellular calcium concentration, the synthesis of many cytokines, and the stimulation of necrosis and apoptosis. Apoptosis may be a significant factor in cell death and may help remove damaged cells Koyner et al., **[30]**.

The current research suggests that Ge-induced renal structural damage and decreased tissue antioxidant levels in rats can be restored by administering aqueous Sa extract at a level of 80mg/kg daily. These results propose that Sa antioxidant

and antiapoptotic properties have a role in these results. Sa extract has been shown to have a general protective effect against renal ischemia/reperfusion in rat models. Malondialdehyde (MDA) levels are significantly reduced by Sa, suggesting its role in ischemia/reperfusion injury Hossein zadeh et al. [10]. In agreement with Goyal et al. [31], pretreatment with Sa reduced the cardiotoxicity caused by isoproterenol via reducing oxidative stress. El-Beshbishy et al. [32] demonstrated that it reduces oxidative stress by increasing the production of SOD and catalase, two important antioxidant enzymes. This result may be counteracting the reactive oxygen species production induced by Ge.

Numerous previous studies have also documented how Sa extract protects against the nephrotoxicities caused by cisplatin, vancomycin, and ceftazidime in rats Naghizadeh et al. [33] and Bathaie, [34].

The significant increase in urea and creatinine in group III of Ge-treated rats can be considered the cause of tubular necrosis observed in this research. This is consistent with Chetan kumar Acharya et al., [13] and Amin et al., [15] as well.

Our study results demonstrated that Sa administration decreases the levels of blood urea and serum creatinine, most likely because of its capacity to protect cells from ROS damage.

In the present work, Ge increased the gene expression of NF-ιχB and TNF-α, indicating the induction of inflammation. Ge induces inflammation as it activates, increases, and translocates NF-ιχB to the nucleus by reducing the level of NF-ιχB inhibitory protein. NF-ιχB then stimulates the production of proinflammatory cytokines, including TNF-α (Ansari et al., [26]; Katary and Salahuddin, [29]. TNF-α also stimulates vasoconstriction, reduces renal blood flow, infiltrates leukocytes, and finally impairs renal function. It has been reported that NF-ιχB inhibition protects the kidneys against Ge Famurewa et al., [35]; Abdelrahman and Abdelmageed, [36].

Our results indicated the anti-inflammatory properties of Sa; this is consistent with Abou-Hany et al., [37]. Ashrafi et al., [38] reported that Sa aqueous extract can protect the kidney and liver of diabetic rats against damage caused by hyperglycemia-induced inflammation, due to its anti-inflammatory potential.

*Conclusion:*

Sa is recommended as a viable new therapeutic approach as a result of this study found that it has an effective and promising role as a protective and reparative agent on the harm that Ge produces to the renal tubules and glomeruli, both structurally and functionally.

*Conflicts of Interest:*

No conflicts of interest as regardsthe publication of this paper was declared by the authors.

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# الدور الوقائى المحتمل للزعفران ضد السمية الكلوية الناتجة عن استخدام عقار الجنتاميسين فى ذكور الفئران البيضاء البالغة دراسة هيستولوجية ومناعية هيستو كيميائية وكيميائية حيوية

يعتبر الجنتاميسين من الأكثر المضادات الحيوى استخداماً فى الممارسات الطبية، وعلى الرغم من استخدامه بالجرعات الموضى بها، تعتبر السمية الكلوية من ابرز الأثار الجانبية الضارة. يعتبر الزعفران من مضادات الأكسدة والالتهابات، لذلك يهدف هذا العمل ابراز الدور الوقائى للزعفران كمضاد للسمية الكلوية التى يسببها الجنتاميسين فى الفئران. تم تقسيم أربعون فأراً أبيضاً بالغاً إلى ١٠ فئران فى كل مجموعة المجموعة الأولى (تحكم طبيعى)، تلقت ٠.٥ مل من الماء المقطر عن طريق الحقن داخل الغشاء البريتونى يومياً، المجموعة الثانية تلقت ٨٠ مجم/كجم من مستخلص الزعفران المائى عن طريق الحقن داخل الغشاء البريتونى يومياً. المجموعة الثالثة تلقت ٧٠ مجم/كجم من الجنتاميسين عن طريق الحقن العضلى يومياً. وتم إعطاء مستخلص الزعفران المائى مع الجنتاميسين للمجموعة الرابعة. استمر العلاج لمدة ٧ أيام. فحصت عينات الدم وعينات من النسيج الكلى فى فئران جميع مجموعات التجربة. استخدمت عينات نسيج الكلى فى كلا من الدراسة المجهرية الضوئية والهيستوكيميائية المناعية، وكيميائية حيوية لقياس الجينات، واختبارات الدم لقياس نسبة اليوريا والكرياتينين، وتم إجراء التحليلات الإحصائية لجميع النتائج. أظهرت الفئران التى أعطيت الجنتاميسين زيادة فى معدلات اليوريا والكرياتينين بالإضافة إلى تنخر أنوبى حاد، تغيرات فى البنية النسيجية للكلى وترسب ألياف الكولاجين بقوة، وزيادة فى النشاط المناعى وجينات وسطاء التاهبات الأنسجة فى الأنسجة التى تم فحصها ومقارنتها بباقى المجموعات بينما أظهرت الفئران التى أعطيت الزعفران إنخفاضاً فى مستويات اليوريا والكرياتينين فى الدم بالإضافة إلى درجة أقل من التنخر الأنبوبى وتحسين البنيه النسيجية للكلى وتقليل جينات وسطاء التهابات الأنسجة فى أنسجة الكلى. ونستخلص من هذا أن مستخلص الزعفران ساعد على تحسين التغيرات النسيجية والكيميائية والكيميائية التى يسببها الجنتاميسين.