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ORIGINAL ARTICLE

Protective Role of Propolis on Gentamicin nephrotoxicity of Adult Albino Rats: Histological and Immunohistochemical study

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ABSTRACT

Background: Gentamicin-induced renal cortical injury is mainly contributed to oxidative stress and other unsettled mechanisms, consequently the present study evaluates the possible antioxidant protective role of the propolis against this renal tubular insult in adult male rats using histological, immunohistochemical, and morphometric analysis. **Material and Methods:** 45 adult male albino rats were divided into five groups (9 rats for each group): control group, propolis treated group (Propolis group) in which the rats received propolis in a dose of 500mg/kg orally for one month. Gentamicin treated group (Gm group) the rats received intramuscular injection of gentamicin in a dose of 120 mg/kg/day once daily from the 2nd to the 7th day of the experiment then sacrificed. Gentamicin withdrawal group (Gm withdrawal group) in which the rats injected by gentamicin as the 3rd group, but sacrificed after one month from the 1st treatment day. Gentamicin and propolis treated group (Gm + Propolis group), the rats treated with gentamicin as the 3rd group and received propolis in the same dose and route of the 2nd group one-hour post gentamicin injection. **Results:** Significant kidney damage was caused by gentamicin, as demonstrated by elevated levels of urea, creatinine, and oxidative stress indicators (MDA level), reduced levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), and reduced glutathione (GSH). Histological alteration including apoptosis, necrosis and inflammatory indicators such as monocyte/macrophages infiltration as well as accumulation of collagen fibers were detected in the renal cortex. In addition, immunohistochemistry data, showed elevated levels of P53 and TNF- α levels. When propolis and gentamicin were taken together, there was a noticeable drop in urea and creatinine levels as well as a reduction in oxidative stress markers, apoptosis, inflammation, collagen fiber depositional renal tissue restoration. **Conclusion:** The present findings suggest that propolis could improve impairment and damage of the renal structures resulting from administration of gentamicin.

Keywords: Gentamicin, Propolis, Kidney, inflammation, Oxidative stress

INTRODUCTION

Aminoglycoside anti-microbial, particularly gentamicin is broadly utilized within the treatment of mortal bacterial contaminations, especially oxygen consuming Gram-negative microscopic organisms. still, the utility of gentamicin is restricted by its genuine side effects on liver and kidney capacities **(1)**.

Nephrotoxicity convinced by gentamicin could be a complex supernatural occurrence considered by an increment in blood urea and serum creatinine consideration, and extreme renal disappointment. Gentamicin causes liver and kidney damage by inducing cellular reactions through a number of different routes. One of the key mediators of gentamycin toxicity was thought to be reactive oxygen species (ROS) and other free radicles generation **(2).** Most Previous literature reported

that gentamycin increases production of ROS and their metabolite, which play the major role in cellular damage through protein oxidation, lipid peroxidation, and DNA damage **(3).** Moreover, Gentamycin induces release of nuclear factor-kappa B (NF-κB) and mitogen- activated protein kinase (MAPK) **(4).**

It was cited that antioxidant composites xenobiotic antagonistic products, using nectar and propolis for wound healing and treatment since ancient times **(5).**

Propolis, also known as bee cement, is a sticky compound made by honeybees from plant components and exudates that is utilized as a global health cure and a hive defense against disease **(6).**

A few reports have demonstrated that propolis portions hold a wide diapason of pharmacological conduct comparable as antidiabetic, antimicrobial, and antitumoral **(7).** Moreover, it was found that propolis includes a powerful protective activity in liver harms **(8&9)** which makes it a perfect preventative agent against gentamicin antagonistic action**.** Two-fold combinations of food components or their bioactive variables can improve antioxidant status, anti-inflammatory, anticancer, and chemoprevention of certain oxidative push and metabolic infections **(10).**

This study aimed to evaluate the potential protective effect of propolis as an antioxidant against the structural alterations in the renal cortex caused by gentamicin in albino rats using histological, immunohistochemical, and morphometric analysis.

MATERIALS AND METHODS

Husbandry and animals

We used 45 healthy adult male albino rats obtained from Animal House in Anatomy Department, Faculty of Medicine, Benha University Egypt. The animals were divided equally into nine rats in five appropriate ventilated and hygienic cages with a cover of metallic mesh and dimension of 55×45×35 cm3. The animals in each cage were provided with access of reach food and water throughout the research time.

This work was carried out in accordance with the principles and guidelines set by the Ethics Committee of Benha University Faculty of Medicine and the US National Institutes of Health's Guide for the Care and Use of Laboratory Animals. (NIH Publication No. RC 16-8-2023).

Chemicals and animal groups

The chemicals

- **Gentamicin:** obtained from Sigma chemical company as 40mg ampule.

- **Bee propolis:** acquired from the Sigma Chemical Company (USA) in St. Louis, Missouri. (Bio Propolis 400mg capsule).

- **Saline and distilled water:** bought from a local market.

- **Assay kits for Lipid Peroxidation (MDA)**, Glutathione Peroxidase Cellular Activity kits, Superoxide Dismutase Assay Kit II, and Creatinine Kcommercial kits, were obtained from Sigma Aldrich Egypt.

2.2.b Animal groups: Five groups (9 animals for each)

Group 1 (Control group): The animals in this group subdivided into two subgroups:

1a: Included four rats with no medication.

1b: Included five rats received the corresponding vehicle for each treatment in the same volume and the same duration. They received oral distilled water (vehicle for propolis), intramuscular saline injections (vehicle for gentamicin).

Group 2 (Propolis group): Nine rats received propolis, the capsule dissolved in a distilled water and given in a dose of 500mg/kg orally **(11)** from the 1st day of the experiment and for 1 month, then the rats were sacrificed

Group 3: Gentamicin treated group (Gm group): Nine rats received intramuscular injection of gentamycin in a dose of 120 mg/kg/day once daily from the 2nd to the 7th day of the experiment according to the procedure described by **(12).** These rats were sacrificed twenty-four hours after the final treatment.

Group 4: Gentamicin withdrawal group (Gm withdrawal group): Rats in this group were given gentamicin for five days using the same method and dosage as the preceding group. However, a month after the first treatment day, the rats were sacrificed.

Group 5: **Gentamicin and propolis treated group (Gm+ propolis treated group)**: The rats in this group received gentamycin with the same dose and the same route of the 3rd group, they received the propolis in a dose of 500mg/kg orally one hour-post gentamicin injection. And continue to receive the propolis to the end of the month, then sacrificed.

Tissue sampling

At the end of the experiment, the rats were anaesthetized by receiving an intraperitoneal injection of 50 mg/kg thiopental **(13)**. Blood sample (2ml) was taken by puncturing of the left ventricle of the heart. Blood samples were

centrifuged at 5000 rpm for 10 minutes, and the separated sera were then kept at -20 °C for further biochemical analysis. One (right kidney) of both kidneys was separated, then divided into 2 halves. One half was immersed in neutral buffered formalin (10%) for processing of histological and immunohistochemical examinations. The other half was homogenated (by homogenising 1 g of frozen kidney tissues in a 1:3 (w/v) ratio with a Tris-HCl sucrose buffered solution at 4 °C) for estimation of antioxidant and oxidative stress markers.

Levels of renal functions tests (blood urea and creatinine):

The corresponding kits were used in accordance with the manufacturer's instructions for the measurement of serum creatinine and urea using the modified Jaffé method (14).

Estimation of antioxidant and oxidative stress indicators

a. Reduced Glutathione (GSH, a nonenzymatic antioxidant) was calculated using a modified version of **(15)** methodology. At 412 nm, the absorbance was measured. The results were presented as µmol/mg proteins.

b. Malondialdehyde (MDA) Estimated by quantifying MDA rates using the method of **(16).** The method's basic idea was to use spectrophotometry to measure the color that was created when thiobarbituric acid (TBA) reacted with MDA. Kidney tissues were homogenised in 1 ml of Tris/HCl buffer (50 mM; pH 7.5) before being centrifuged for 10 min at 1000 xg.

To do this, 2.5 ml of trichloroacetic acid solution and 0.5 ml of homogenate were mixed, and the mixture was immersed in a boiling water bath for 15 minutes. After cooling, the mixture was added to 1 ml of TBA solution and 2 ml of supernatant. The MDA rate was expressed in terms of µmol/mg proteins, and the solution's absorbance was measured at 533 nm after cooling.

c. Superoxide dismutase (SOD, antioxidant enzymes): was determined as **(17)**. At, the absorbance was measured, the change in optical density was monitored for three minutes at 420 nm. On the basis of its capacity to prevent the aut-oxidation of pyrogallol32, the SOD activity was assessed. In a nutshell, 2.80 ml of Tris succinate buffer (0.05 M, pH = 8.1) was added to 50 µl homogenate. In order to start the reaction, 100 µl of 8.0 mM pyrogallol were added. For three minutes, the change in absorbance at a wavelength of 420 nm was monitored every 30 seconds. For comparison, a reaction mixture containing homolysate was replaced with 50 µl of pure water. Units of SOD activity per milligram of protein were used to measure it.

d. Glutathione peroxidase (GPx, antioxidant enzymes): was carried out utilizing the procedure described in (**17**). By detecting changes in absorbance at 340 nm brought on by the consumption of NADPH in the existence of H2O2, GSH, and glutathione reductase, the glutathione peroxidase activity was calculated. The results were represented as IU/mg protein.

Histopathological and immunohistochemistry study

The kidneys that had been fixed in formalin were ready for paraffin microoperations. Involving dehydrating them in ethanol solutions of ascending grades, clearing them with xylene, infiltrating them with paraffin, and finally sectioning them. Five micron-thick kidney sections were stained with Hematoxylin and Eosin (H & E), Masson Trichrome staining [18], and immunohistochemistry staining [19] for detection of P53 and TNF-α immunoexpressions. All the used markers were rabbit polyclonal primary antibodies (Catalog #: ab131442, and A11534 respectively). P53 was purchased from Abcam, Cambridge, United Kingdom while, TNF-α was from AB clonal Inc., Woburn, MA, USA. For antigen retrieval, the sections were boiled for 10 min in 6 citrate buffer (Catalog #: AP9003-125, Epredia, Thermo Fisher Scientific) at pH 6. After that and for 1 hour, the renal sections were incubated with the primary antibodies (at dilutions 1:100, and 1:200 for P53and TNF- α respectively). The detection system used for completing the immunoreaction was rabbit-specific kits (TP-015-HD, Lab Vision™, Thermo Fisher Scientific). A chromogen, 3,3-diaminobenzidine (DAB), and hematoxylin counterstaining were used. The positive reaction appeared as brownish cytoplasmic discoloration (for TNF- α) and positive nuclear (for P53). Slide visualization and image photographing were performed in the Anatomy Department, Faculty of Medicine, Benha University, Egypt. For such purpose, Nikon Eclipse 80i upright microscope (Nikon Corporation, Japan) with a fitted digital camera, Toup Cam TM Xcam full HD camera (ToupTek Europe, Ultramacro Ltd., UK) was used.

Morphometric study

Three rats from each group were randomly selected for measurements in five non-overlapping fields photographs. At a magnification of x400, images were assessed using Image J software (version 1.52, Public Domain) (**20)**.

The average area % of collagen fibers deposited in Masson's trichrome-stained sections and TNF-immunoreaction and the area % of P53 were assessed

To guarantee a blind approach, image study was carried out by another person who was a subject matter expert and was not aware of the experimental groups.

STATISTICAL ANALYSIS

The statistical analysis was performed with IBM SPSS v27, Armonk, NY, USA. Histograms and the Shapiro-Wilk test were used to determine whether the data distribution was normal. Both the ANOVA (F) test and the post hoc Tukey test were used to assess quantitative parametric data. The standard deviation (SD) and mean of the results were calculated. The P value of a result was deemed statistically significant if it was less than 0.05.

RESULTS

Group 3 treated with gentamicin exhibited a substantial ($p<0.05$) rise in urea concentration. This increase was also highly significant ($p<0.05$) when compared to group 2 treated with propolis alone and group 1 that received no treatment. When gentamycin-treated group 3 was compared to groups 1 and 2, the creatinine level showed a substantial increase ($p<0.05$). However, there was a highly significant difference ($p<0.05$) when group 4 and the combined group 5 were compared to group 3. (Table 1) (Fig 1&2).

Table 1: Estimation of kidney function tests (blood urea & creatinine) of the studied groups

	Group 1 (n=9)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)	Group 5 (n=9)	P value
Urea (mg/dl)	25.8 ± 1.86	27.1 ± 1.83	40.2 ± 3.53	36.3 ± 2	30.2 ± 1.3	**<0.001***
	P1	0.713	**<0.001***	**<0.001***	**0.001***	
	P2		**<0.001***	**<0.001***	**0.039***	
	P3			**0.006***	**<0.001***	
	P4				**<0.001***	
Creatinine (mg/dl)	0.6 ± 0.15	0.7 ± 0.15	1.8 ± 0.23	1.5 ± 0.23	1.1 ± 0.22	**<0.001***
	P1	0.765	**<0.001***	**<0.001***	**<0.001***	
	P2		**<0.001***	**<0.001***	**<0.001***	
	P3			**0.009***	**<0.001***	
	P4				**0.005***	

Data are presented as mean ± SD. *: Significantly different as P value ≤0.05. P1: P value compared to Group 1, P2: P value compared to Group 2, P3: P value compared to Group 3, P4: P value compared to Group 4.

Table 2: Estimation of antioxidant and oxidative stress indicators & markers of the studied groups

	Group 1 (n=9)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)	Group 5 (n=9)	P value
Reduced glutathione (µmol/mg proteins)	4.1 ± 0.51	3.7 ± 0.58	1.5 ± 0.43	2.2 ± 0.63	3 ± 0.43	**<0.001***
	P1	0.461	**<0.001***	**<0.001***	**<0.001***	
	P2		**<0.001***	**<0.001***	**0.037***	
	P3			**0.033***	**<0.001***	
	P4				**0.037***	
Malondialdehyde (µmol/mg proteins)	1.3 ± 0.48	1.7 ± 0.43	5.3 ± 1.21	4 ± 1.2	2.9 ± 0.53	**<0.001***
	P1	0.827	**<0.001***	**<0.001***	**0.002***	
	P2		**<0.001***	**<0.001***	**0.043***	
	P3			**0.025***	**<0.001***	
	P4				**0.043***	
Superoxide dismutase (IU/mg proteins)	78.4 ± 4.42	73.1 ± 3.98	40.7 ± 5.02	47.9 ± 5.21	66.9 ± 4.08	**<0.001***
	P1	0.117	**<0.001***	**<0.001***	**<0.001***	
	P2		**<0.001***	**<0.001***	**0.046***	
	P3			**0.014***	**<0.001***	
	P4				**<0.001***	
Glutathione	277.9 ± 7.96	272 ± 9.97	203.1 ± 8.52	215.4 ± 10.51	259.3 ±	**<0.001***

peroxidase (IU/mg proteins)					6.58
	P1	0.621	**<0.001***	**<0.001***	**<0.001***
	P2		**<0.001***	**<0.001***	**0.032***
	P3			**0.038***	**<0.001***
	P4				**<0.001***

Data are presented as mean ± SD. *: Significantly different as P value ≤0.05. P1: P value compared to Group 1, P2: P value compared to Group 2, P3: P value compared to Group 3, P4: P value compared to Group 4.

Table 3: Percentage area of collage fibers deposition in the renal tissues in the studied groups

		Group 1 (n=9)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)	Group 5 (n=9)	P value
Masson (%)	**Mean ± SD**	9.2 ± 0.67	7.7 ± 0.5	20 ± 1.5	18 ± 2	11.3 ± 1.22	**<0.001***
	Range	8 – 10	7 – 8	18 – 22	15 - 20	10 – 13	
	P1		0.102	**<0.001***	**<0.001***	**0.011***	
	P2			**<0.001***	**<0.001***	**<0.001***	
	P3				**0.018***	**<0.001***	
	P4					**<0.001***	

Data are presented as mean ± SD. *: Significantly different as P value ≤0.05. P1: P value compared to Group 1, P2: P value compared to Group 2, P3: P value compared to Group 3, P4: P value compared to group 4.

Table 4: Percentage area of TNF-α & P53 of the studied groups

	Group 1 (n=9)	Group 2 (n=9)	Group 3 (n=9)	Group 4 (n=9)	Group 5 (n=9)	P value
TNF-α	12.7 ± 2.18	13.7 ± 1.66	24.2 ± 2.39	20.2 ± 2.91	16.9 ± 2.26	**<0.001***
	P1	0.889	**<0.001***	**<0.001***	**0.003***	
	P2		**<0.001***	**<0.001***	**0.039***	
	P3			**0.006***	**<0.001***	
	P4				**0.031***	
P53	0.9 ± 0.22	1.1 ± 0.21	4.5 ± 0.89	3.3 ± 1.05	2 ± 0.62	**<0.001***
	P1	0.984	**<0.001***	**<0.001***	**0.011***	
	P2		**<0.001***	**<0.001***	**0.042***	
	P3			**0.007***	**<0.001***	
	P4				**0.003***	

Data are presented as mean ± SD. *: Significantly different as P value ≤0.05. P1: P value compared to Group 1, P2: P value compared to Group 2, P3: P value compared to Group 3, P4: P value compared to Group 4. TNF-α: Tumor necrosis factor alpha.

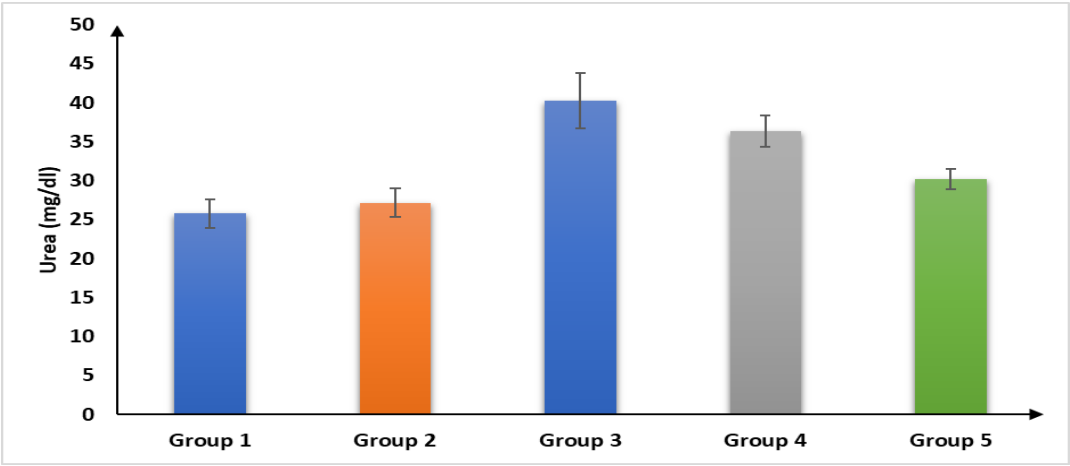

Figure 1: Estimation of blood urea of the studied groups

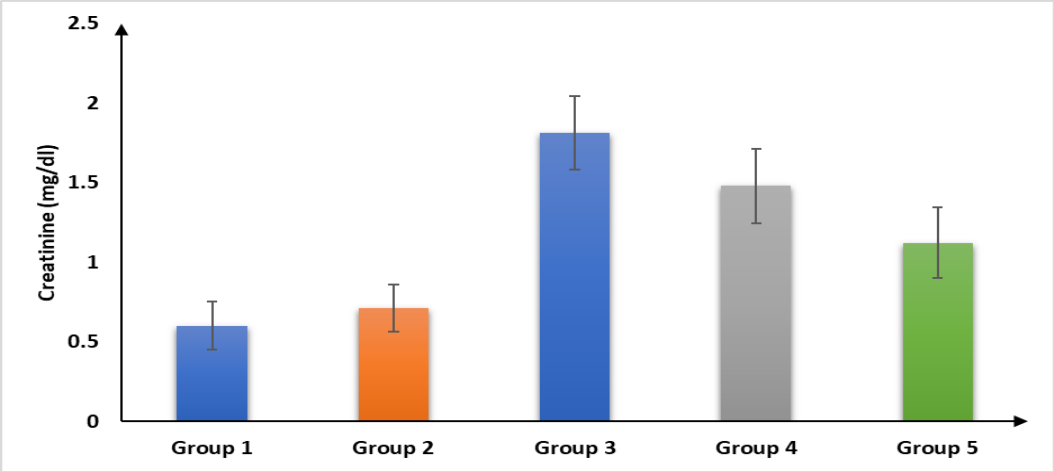

Figure 2: Estimation of blood creatinine of the studied groups

Comparing group 3 to groups 1 and 2, the Gm treated group exhibited a statistically significant rise in malondialdehyde (MDA) and a substantial drop in reduced glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels ($p<0.05$). When compared to Gm treated group 3, there is a significant decrease in MDA levels and an increase in other antioxidant enzymes in both Gm withdrawal group 4 and Gm+ propolis treated group 5 ($p<0.05$). (Table 2) (Fig 3-6).

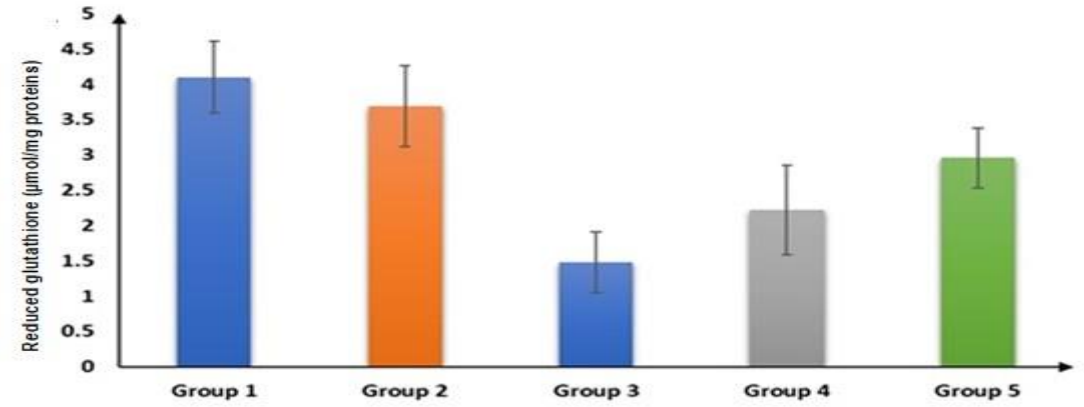

Figure 3: Estimation of reduced glutathione of the studied groups

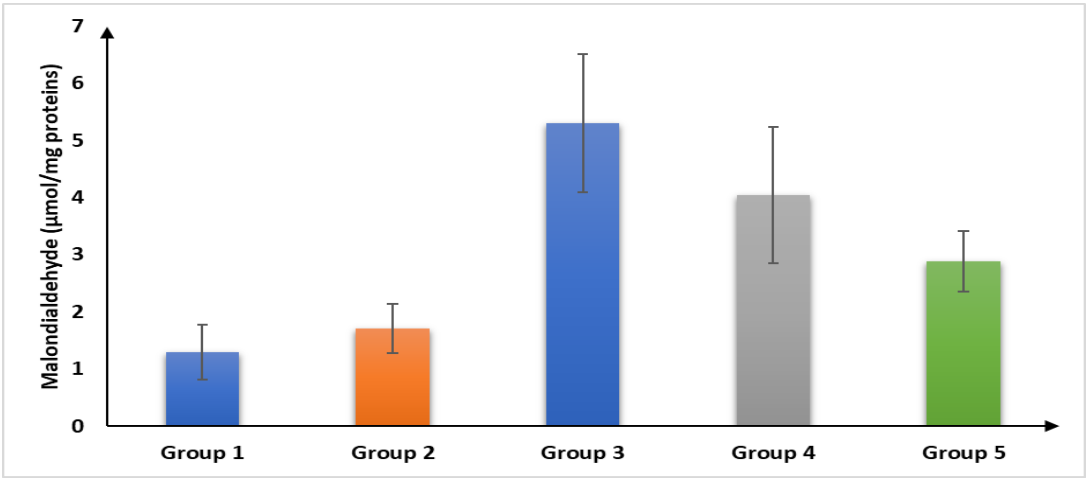

Figure 4: Estimation of malondialdehyde of the studied groups

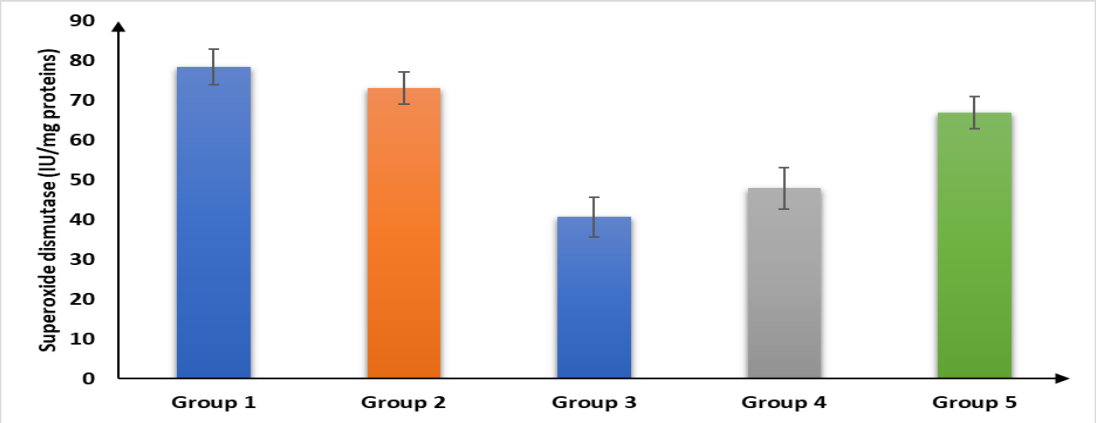

Figure 5: Estimation of superoxide dismutase of the studied groups

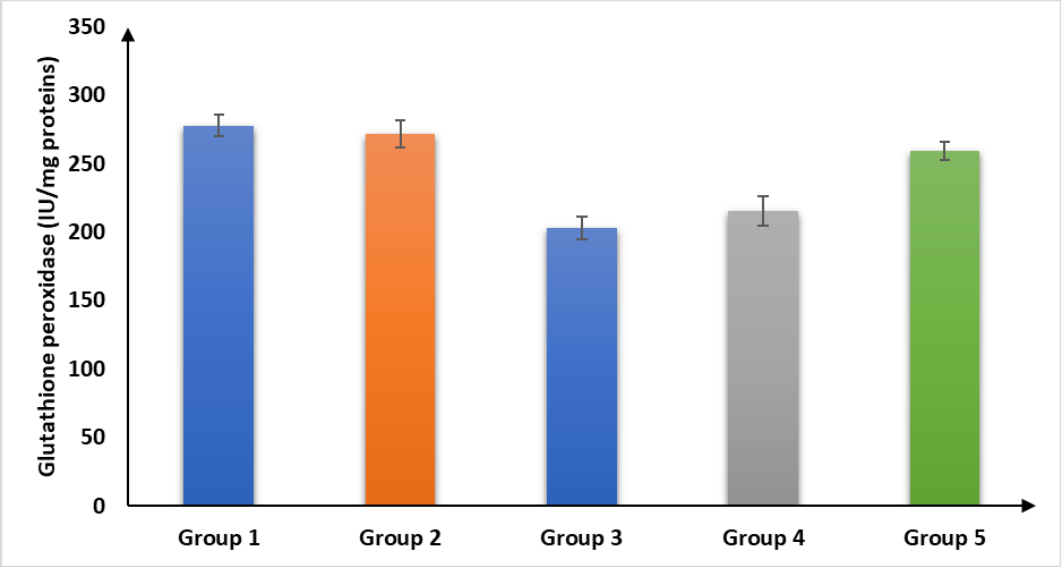

Figure 6: Estimation of glutathione peroxidase of the studied groups

The analysis of kidney-stained sections with hematoxylin and eosin (H&E) from the control subgroups (Figure 7a) and propolis alone group 2 (Figure 7b) revealed normal proximal and distal convoluted tubules as well as renal corpuscles in the renal cortex. The glomerulus contained capillary tuft and mesangial cells, surrounded by an impervious capsule denominated Bowman's capsule. Modified epithelial cells called podocyte line the parietal layer of this capsule. The proximal convoluted tubules (PCT) have a narrow lumen lined by simple cuboidal epithelium characterized by a brush border of its microvilli. The distal tubules (DCT) showed wide lumina surrounded by cuboidal cells featuring nuclei that were rounded and vesicular in shape, along with cytoplasm that displayed a mild acidophilic nature.

Rats from Gm treated group, revealed marked tubular and glomerular changes (Figure 7c). Glomerular changes appeared in the form of marked disturbance in its architecture, degenerated mesangial matrix with glomerular atrophy leading to widening of capsular space and loss of its podocyte. inflammatory cells deposition also noticed. Tubular changes appeared in form of degeneration up to necrosis, which included both the distal and proximal tubules. Degenerated tubules showed dilatation, cytolysis, desquamation of lining epithelium, intraluminal hyaline deposition in some tubules and loss of brush border of proximal tubules.

Sections from the Gm withdrawal and Gm + propolis treated groups showed noticeable restoration of the normal renal tissue which is more visible in the combined group 5 than gentamycin withdrawal group 4 (Figure 8 a &b).

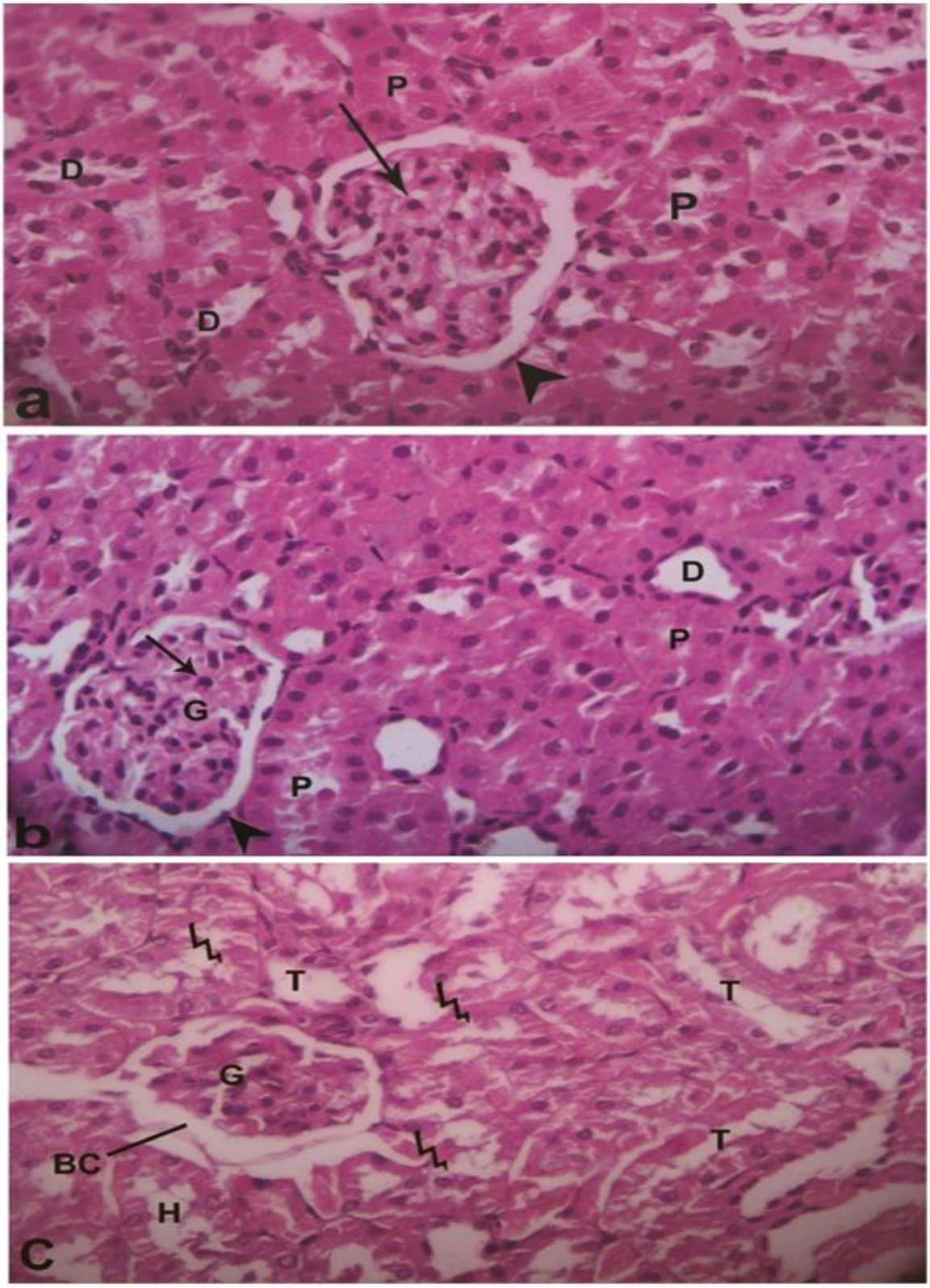

Figure 7 (a&b&c): Photomicrographs of control group I **(a)** and propolis treated group II **(b)** showing: Glomeruli (G) with its typical mesangial cells (Arrow). Surrounded by Bowman's capsule (Arrow head)) with its lining podocyte, proximal convoluted tubules (P) and distal convoluted tubules (D). **(c)** A photomicrograph of Gm treated group showing: atrophied glomeruli (G), wide capsular space (Bowman's space) (Bc) with loss of its podocytes, wide renal tubules (T) with desquamation of its lining epithelium (zigzag arrows) and intraluminal hyaline deposition (H). (H&E X 400).

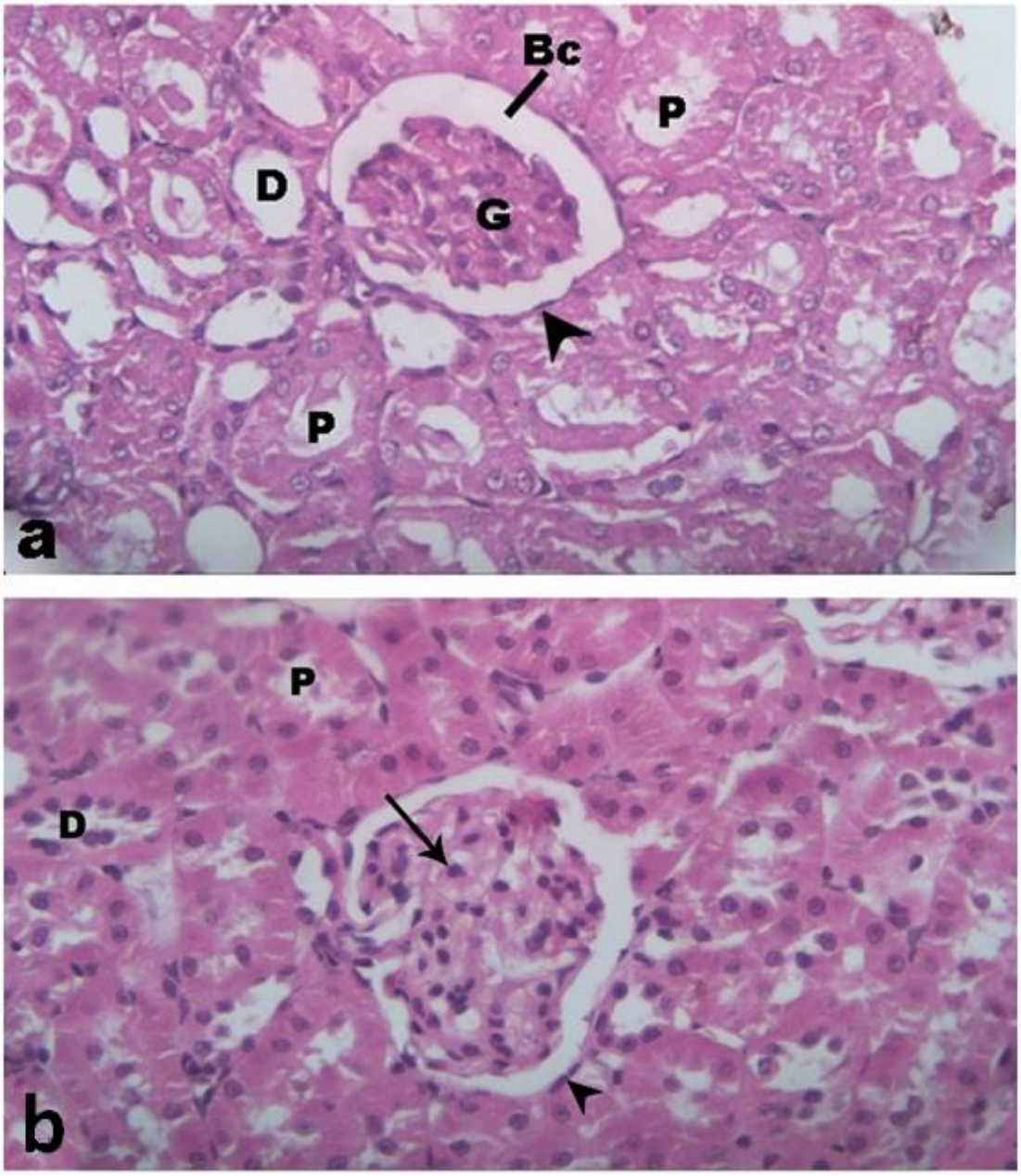

Figure 8 (a & b): Photomicrographs of Gm withdrawal group **(a)** and Gm+ propolis treated group **(b)** showing: Glomeruli (G) with their mesangial cells(Arrow) surrounded by the podocytes and Bowman's space (Bc) and Bowman's capsule (arrow head). Proximal convoluted tubule (P) and distal tubule (D). (H & E X 400).

When sections from the propolis-treated and control groups were stained with Masson trichrome-stain, they revealed no fibrotic alterations and a similar arrangement of collagen fibers inside the glomeruli and between the tubules (Figure 9a&b). In sections from the 3rd group, there was a widespread bluish discoloration denoting diffuse collagen deposition inside the glomeruli with thickening of its basement membrane, between the tubules and on their brush border (Figure 9c). In both 4th and 5th groups, there is minimal regress in the collagen deposition inside the glomeruli and the interstitial tissue. (Figure 10a&b).

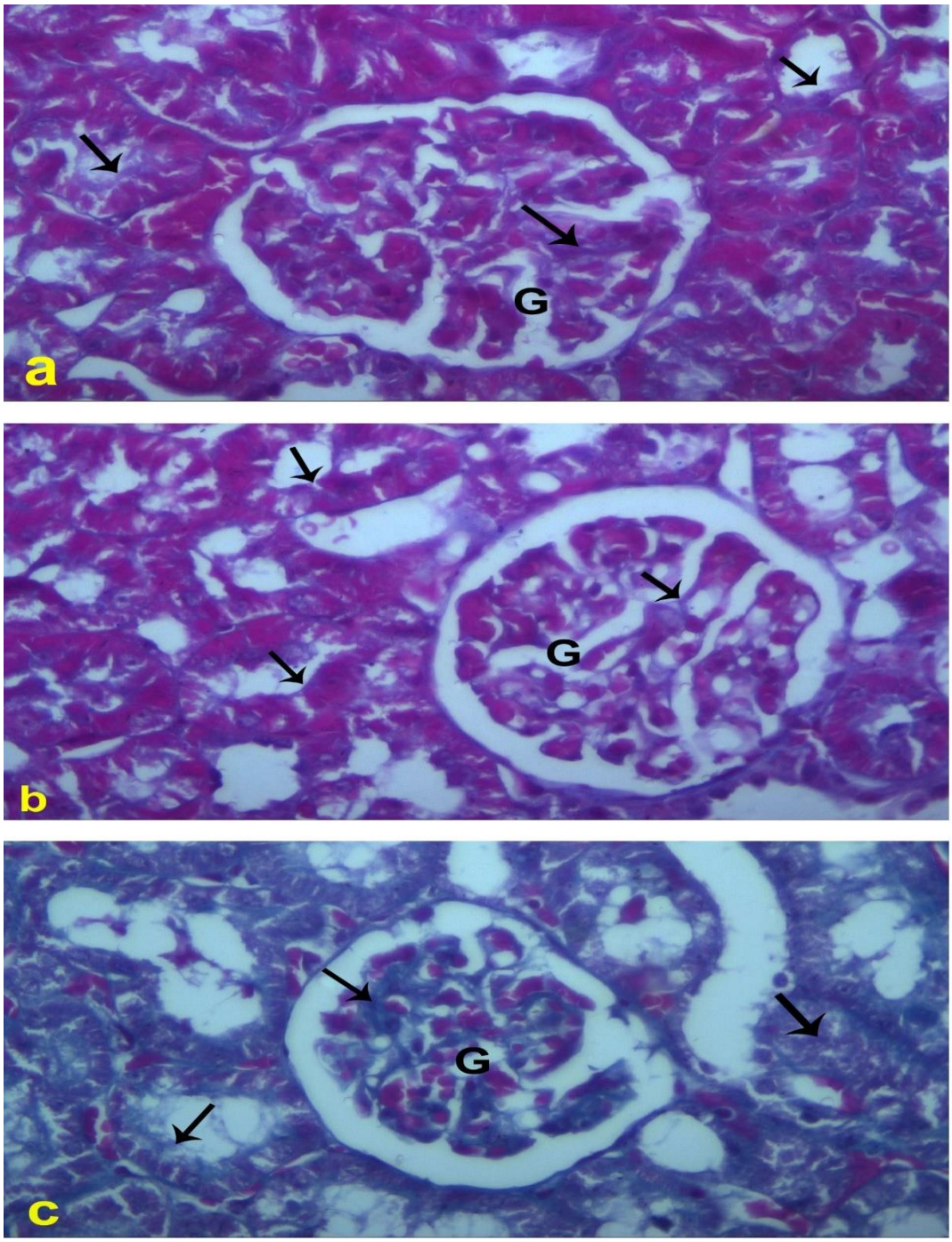

Figure 9 (a&b&c): Photomicrographs of control group I **(a)** and of propolis treated group II **(b)** showing: Little distribution and density of the collagen fibers (blue-stained) inside the glomeruli (G) and on the brush border of the tubules (arrows). **(c)** A Photomicrograph section of Gm treated group showing: widespread and dispersed collagen deposition inside the glomeruli (G) and in the tubules on the brush border (Arrow). (Masson's trichrome X 400).

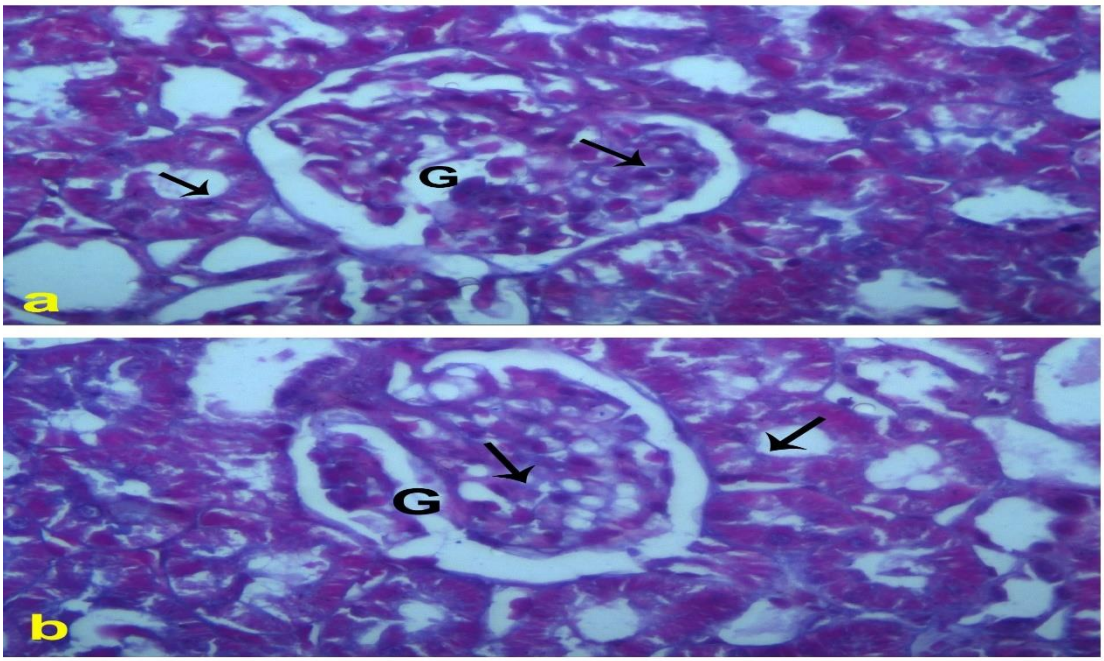

Figure 10 (a&b): Photomicrographs of Masson's trichrome stained renal tissue sections of Gm withdrawal group **(a)** and Gm and propolis treated group **(b)** showing: Observed decrease in staining blue intensity in the glomeruli (G) and on the brush border of the tubules (arrow), suggests a low presence of collagen. (Masson's trichrome X 400).

When gentamycin treated group 3 was compared to control group 1 and propolis treated group 2, the mean area percentage of collagen fibers deposition in sections stained with Masson in the renal cortex increased significantly ($p<0.05$). There was a significant decrease in collagen fiber content in both the Gm withdrawal and Gm+ propolis treated groups as compared to the Gentamycin treated group 3 ($p<0.05$). (Table 3) (Fig 11).

Group 4.

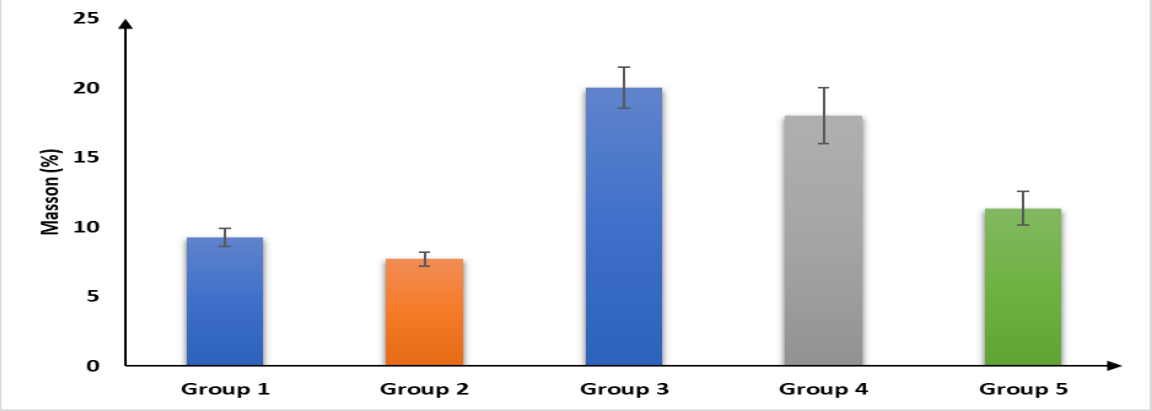

Figure 11: Percentage collage fibers deposition in the renal tissues of the studied groups

The result of P53 immunoreaction reversed absence of a nuclear reaction in control group (Figure 12a) and the group receiving propolis only (Figure 12b) where they had similar outcomes. In comparison with the control group, the Gm-treated group exhibited a significant and extensive distribution of P53 immunoreactivity, as depicted in (Figure 12c). The immunoreactivity of P53 in both Gm withdrawal and Gm with propolis treated groups (Figure 13a&b), The nuclear immunoreaction was decreased to high extend.

Immunohistochemical observations of TNF-α showed marked increase immunopositivite cytoplasmic reaction in group 2 (Figure 14c) as compared to the cytoplasmic reaction in 1st and 2nd groups (Figure 14a&b). Meanwhile, there is restoration of TNF-α immunoreactivity in both Gm withdrawal (Figure 15a) and Gm with propolis co-treated group (Figure 15b).

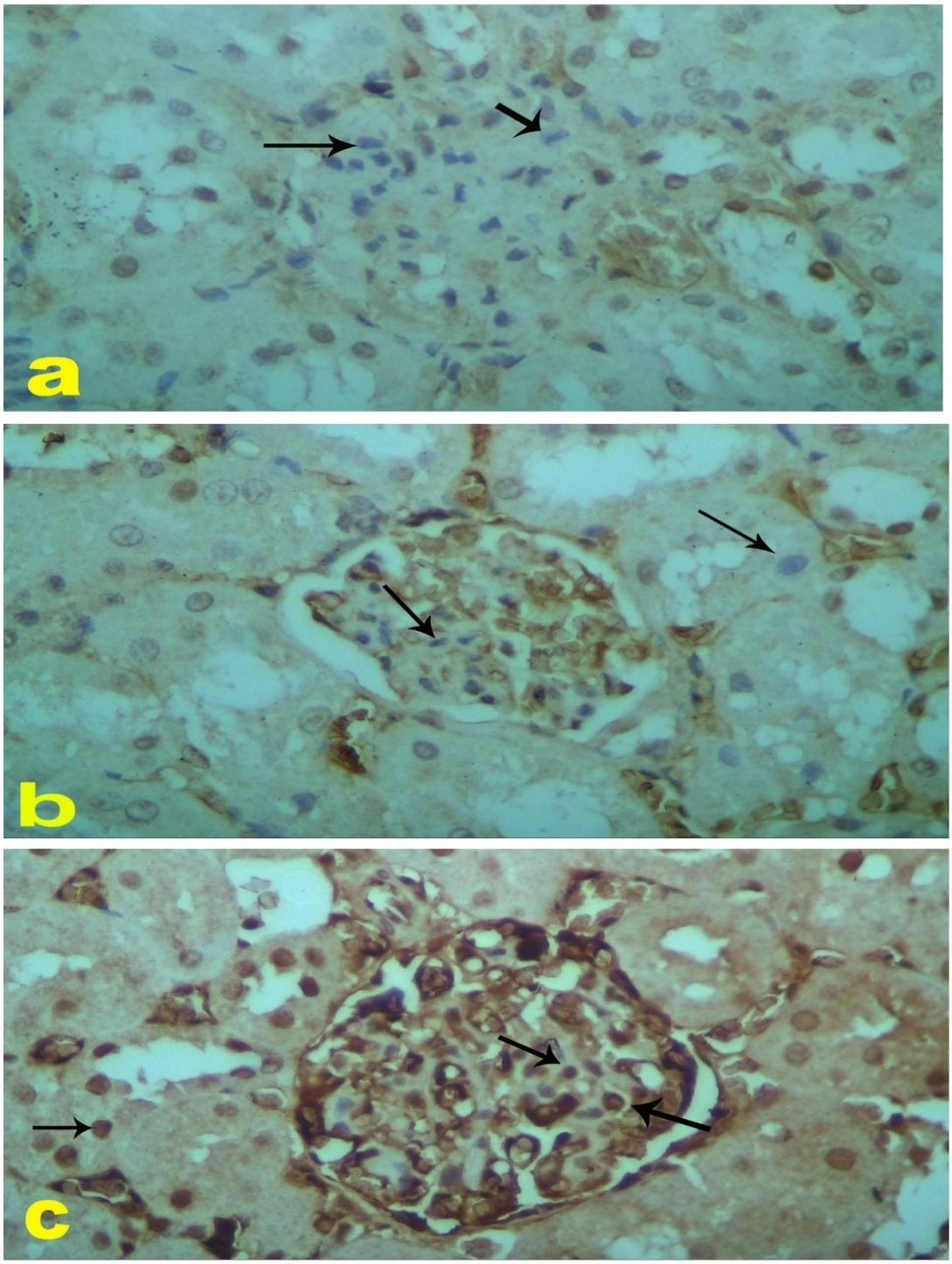

Figure 12 (a&b&c): Photomicrographs of P53 immunoreactivity renal sections of control group **(a)** and propolis alone-treated group **(b)**: Both groups show negative nuclear P53 immunoreaction (Arrows). **(c)** Photomicrograph of P53 immunoassay renal section of Gm treated group showing marked positive nuclear reaction (arrows)**. (P53 X 400).**

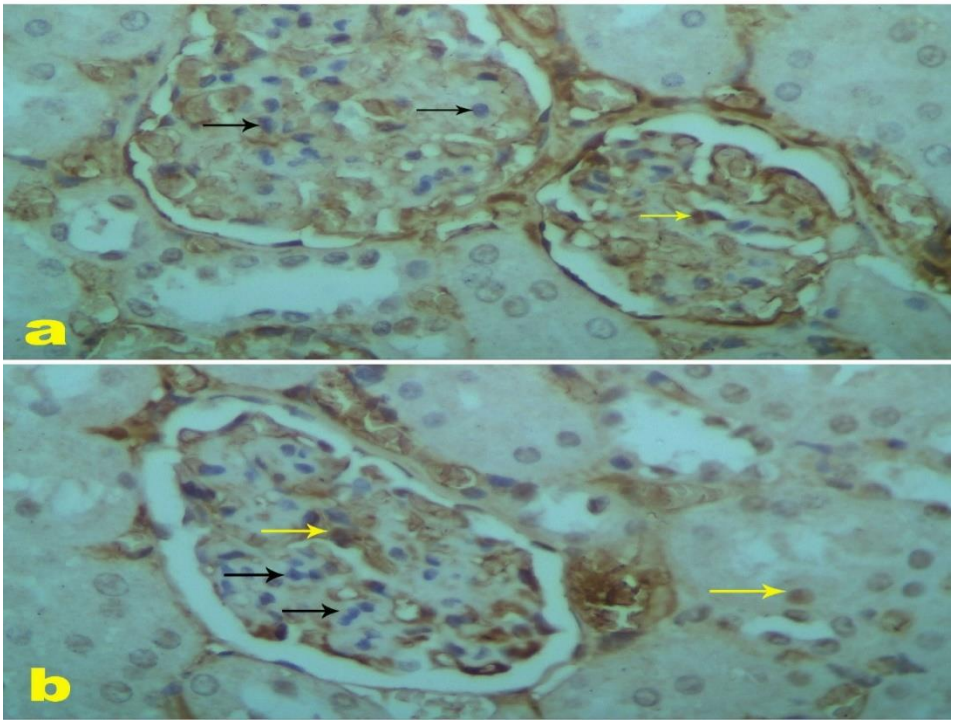

Figure 13 (a&b): Photomicrographs of P53 immunoreactivity renal sections of the 4th group **(a)** and 5th group **(b)** showing: Mild positive nuclear reaction, where there are some negative nuclei (black arrows), and other positive nuclei (yellow arrow). (P53 X 400)

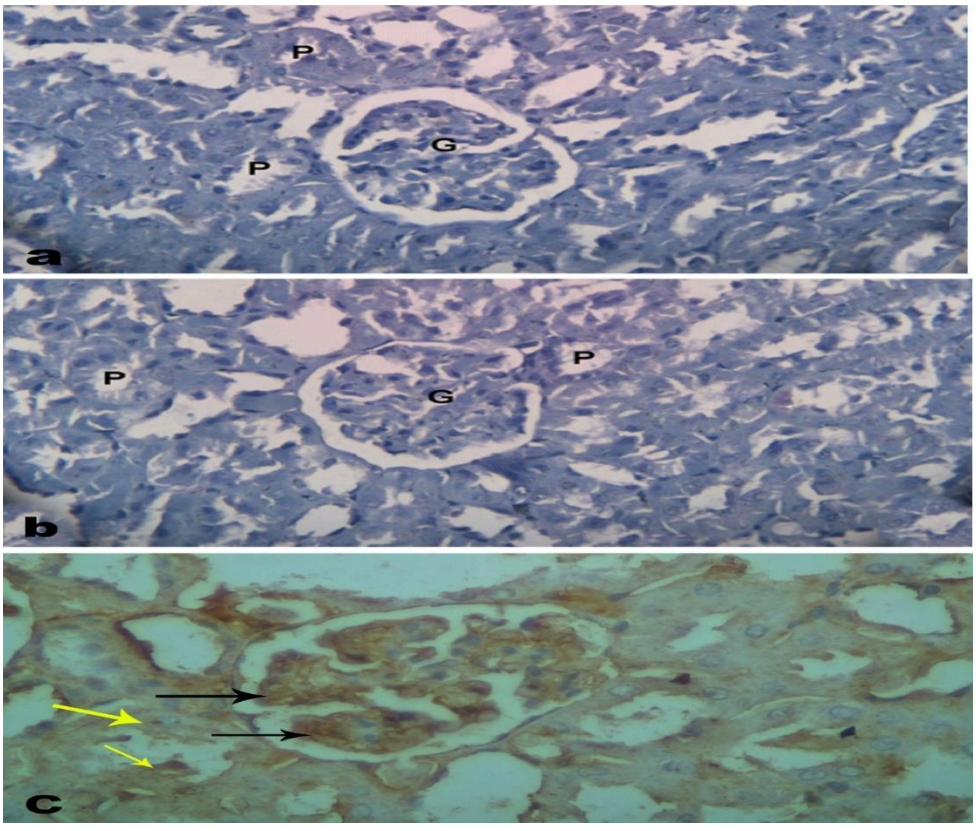

Figure 14 (a&b&c): Photomicrographs of TNF-α immunostained renal sections of control group **(a)** and Propolis alone treated group **(b)** showing: Negative cytoplasmic immunoreaction in the glomeruli (G) and proximal tubules (P). **(C)** Photomicrograph of TNF-α immunostained renal section of Gm treated group showing: Marked TNF-α cytoplasmic immunopositivity in the mesangial cells (black arrow) and renal tubules (yellow arrows). (TNF- α X 400).

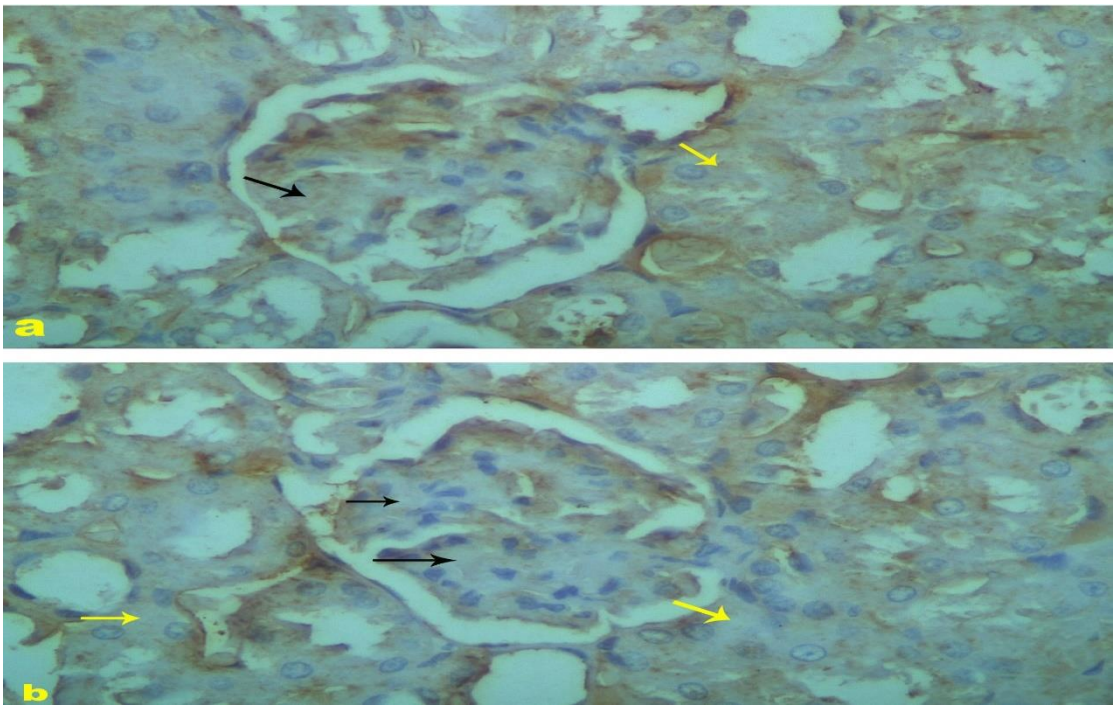

Figure 15 (a&b): Photomicrographs of TNF-α immunostained renal sections of Gm withdrawal group **(a)** and Gm + propolis co-treated group **(b)** showing: The cytoplasm of mesangial cells (black arrow) and renal tubules (yellow arrows) with an obvious decrease in the TNF-α immunoreactivity. (TNF- α X 400).

Group 3 that received gentamycin treatment exhibited a noteworthy rise in TNF-α and P53 levels ($p < 0.05$) when compared to groups Gm withdrawal 4 and Gm+ propolis 5, and a highly significant increase ($p < 0.05$) when compared to groups 1 and 2 that received propolis alone treatment. TNF-α and P53 levels were significantly lower in the Gm+ propolis 5 group than in the Gm withdrawal 4 group ($p<0.05$). (Table 4) (Fig 16&17).

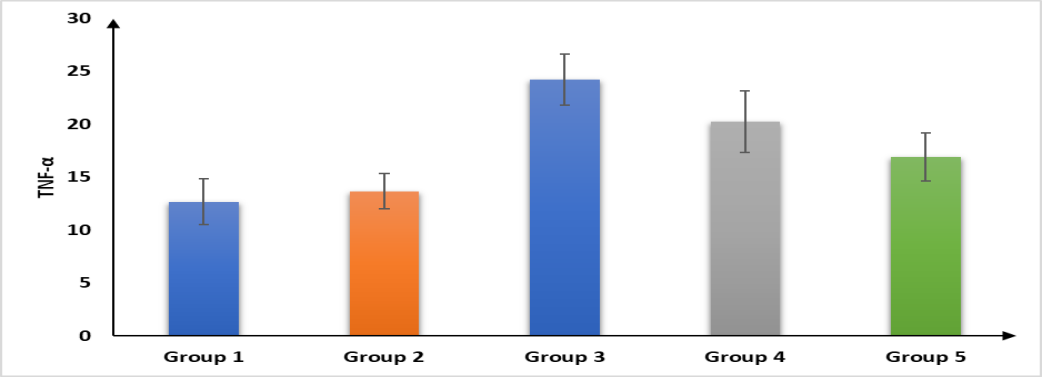

Figure 16: TNF-α of the studied groups

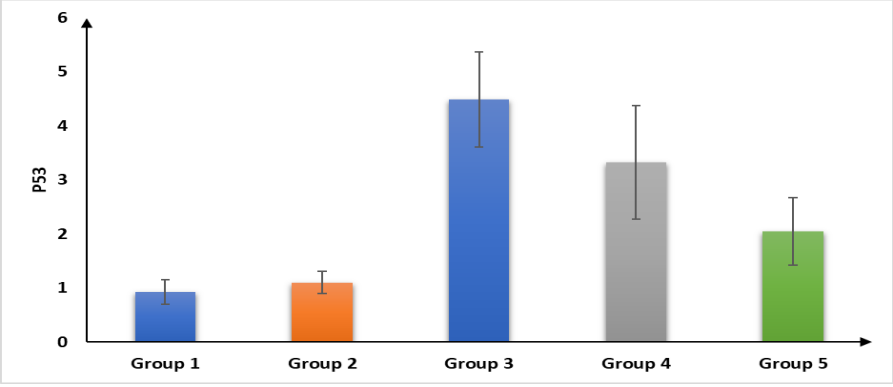

Figure 17: P53 of the studied groups

DISCUSSION

It is well known that aminoglycoside antibiotics, and particularly the most widely used one, gentamicin, are nephrotoxic **(21).** According to a number of studies, oxygen-free radicals are thought to be significant mediators of acute renal failure generated by gentamicin (Gm). Accordingly, using substances with strong antioxidant qualities is one of the primary strategies used to reduce Gm-induced nephrotoxicity. According to a number of recent studies, propolis or any of its constituents may be helpful in reducing Gm nephrotoxicity symptoms **(22&23).**

In this work, Gm (120 mg/kg/day) was injected once daily for five days to cause acute nephrotoxicity. Propolis (PR) (500 mg/kg orally for one month) was employed as a strong antioxidant and free-radical scavenger to mitigate the oxidative damage and renal damage caused by Gm, That was supported by Laaroussi et al **(23)** who found that the acute nephrotoxicity was created by injecting Gm (120 mg/kg BW/day, i.p.) In this study, we employed Propolis, a strong antioxidant and free-radical searcher, to mitigate the oxidative damage and kidney impairment caused by Gm.

Plasma creatinine concentration is a more potent marker in the early stages of renal disease than urea concentration. Furthermore, injury to parenchyma tissue is the only way that urea concentration rises. **(24&25).**

The main findings of our study indicate that the administration of Gm causes higher levels of creatinine and urea when compared to the control group. On the other hand, after stopping Gm and starting PR medication, the creatinine and urea levels were considerably lower than those of the Gm group. Regarding this, Abdelrahman and Abdelmageed **(26)** demonstrated that, Gentamicin contributed to a significant ($p < 0.05$) increase in serum creatinine and urea level when compared to the control group.

Furthermore, Atta et al **(27)** demonstrated that, the Gm group's plasma creatinine and urea levels were higher than the control group ($p<0.05$). Thus, it is believed that renal impairment is indicated by the elevated blood urea and creatinine levels in rats getting Gm therapy. This outcome concurs with what was reported by **(28&29&30)** .

Gm-induced oxidative stress causes lipid peroxidation, raising MDA levels and lowering antioxidant enzymes like superoxide dismutase and catalase (CAT) **(31).** Propolis treatment prevents gentamicin-induced CAT activity depletion, potentially preventing hydrogen peroxide damage in renal cell lines **(28).**

The study found that Gm-induced oxidative stress reduces antioxidant enzymes like catalase and superoxide dismutase, which are used to track oxidative stress-related renal tubular injury **(24).** Propolis's protective action against gentamicin-induced decrease in these enzymes may have facilitated the restoration of indicators of renal tubular injury, as it may have reduced malondialdehyde levels in the Gm group compared to the control group **(23).** Since propolis has been shown to reduce nephrotoxicity in renal experiments using gentamicin **(23)** and **(27),** amikacine **(28)** and doxorubicin **(32)** it is plausible to believe that it has this ability.

According to histopathological studies, Gentamicin intake in a dose of (120 mg/kg/day, once daily for five days) can significantly alter kidney structure, causing degeneration and necrosis in both proximal and distal tubules, glomerular architecture disturbances, and a widening of the renal corpuscle due to glomerular atrophy.

Some researchers published histopathological results and structural alterations in renal tissue caused by aminoglycoside antibiotics like Gm **(33&1).** According to Atta et al. **(27)** the administration of Gm caused alterations in the tubular and glomerular epithelium.

The therapy with propolis has shown significant restoration of normal renal tissue, renal corpuscles, and tubules, with some degenerated tubules, suggesting it could reverse Gm toxic effect, and preserving collagen fiber distribution

According to research by Sahu et al. **(34),** gentamicin causes glomeruli and tubules to undergo apoptosis. However, propolis was also administered at the same time to lessen the toxicity. Teles et al. have also shown the renoprotective action of Brazilian red propolis **(35).** Furthermore, Aldahmash et al **(36)** reported that the kidney tubules had significant changes as a consequence of the gentamicin injection. The degeneration and necrosis of the tubule epithelial cells may have resulted from the reabsorption of gentamicin in the proximal convoluted tubules causing dilated tubules, tubular degeneration, substantial leucocytic infiltrations, loss of the

brush border, and the formation of tubular casts. These results concur with those of **(37).**

When propolis and gentamicin were taken together, the renal tubules significantly improved as evidenced by the lack of tubular casts, a decrease in infiltration, degeneration, and tubular dilatation. Similar results with propolis co-administration leading to normal epithelial lining with brush boundaries in proximal convoluted tubules were also described by Azab et al **(38).** On the other hand, some tubules with disturbed brush boundaries seemed to be renewing.

The exact processes underlying nephrotoxicity induced by gentamicin remain unclear. Nonetheless, the pathogenic processes of nephrotoxicity that have been suggested include the production of oxidative stress, apoptosis, necrosis, and the growth of the monocyte and macrophages.**(28).**

The investigation revealed a market increase in collagen fibers in glomeruli and renal tubules in sections treated with gentamicin, as assessed using Masson's trichrome stain. This was in agreement with Aldahmash et al. **(28)** who observed that under the influence of growth factors and inflammatory cells, gentamicin therapy causes a notable deposition of collagen fibers in the glomeruli and cortical tubules of mice, encouraging myofibroblasts to form extracellular matrices. Regarding the increased levels of tumor necrosis factor-α caused by gentamicin therapy, it contributes to the production of myofibroblasts by the conversion of interstitial cells and the subsequent deposition of collagen. **(39).**

Apoptosis is shown to play a major role in several renal disorders, drug-promoted nephrotoxicity, and human kidney physiological functioning **(40).** Because it prevents the formation of aberrant cells, p53, a tumor suppressor, is thought to be an important mediator of cell death **(41)**. P53 likewise plays a major role in mitochondrial-related apoptosis, which is the mechanism by which gentamicin induces cell death **(42).** In contrast, active immune cells release TNF-α, an innate danger signal, in response to inflammation. **(43).**

P53 immunoreactivity was found to be significant in the GEN-treated group, while GM withdrawal and propolis treatment diminished P53 reactivity.

TNF-α in propolis-alone and control groups had negative reactions, but with Gm treatment, the immunoreaction increased. Consistent with our findings, earlier research demonstrated elevated p53 protein expression levels in rats treated with gentamicin **(34&44)**. Also, Geyikoglu et al **(45)** showed Gm administration causes increased P53 level. Additionally, Laaroussi et al **(23)** demonstrated that, rat kidney MDA levels, TNF-α expressions, and pro-apoptotic protein were significantly reduced by propolis (200 mg/kg).

CONCLUSION

Gentamicin injection causes renal glomeruli and tubule degeneration, with histological and immunohistochemical studies showing gentamicin withdrawal has limited effect. Propolis administration improves these changes.

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