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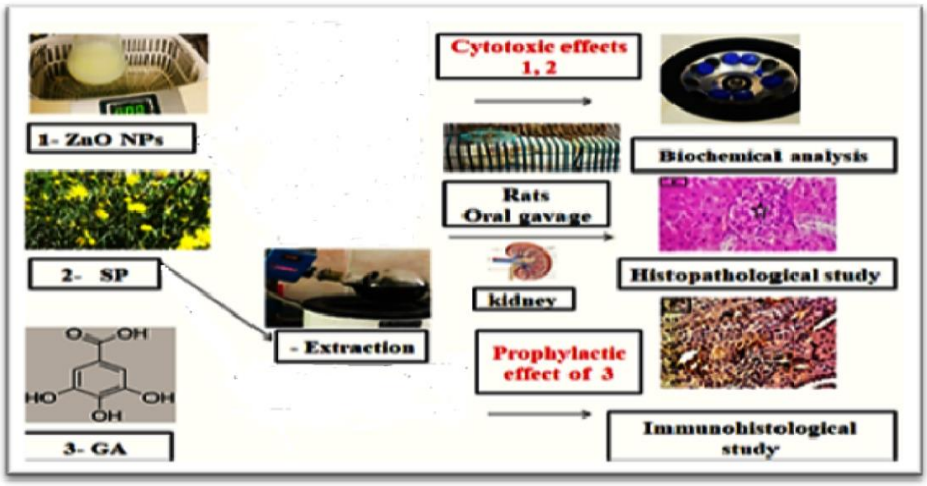
Cytotoxic effects of zinc oxide nanoparticles and ethanolic extract of mureer plant in renal tissue via apoptosis mechanism induction with the hopeful beneficial role of gallic acid in rats

Nouran EL-Shafey[1], Ali El-Shekih[1], Nabil Moustafa[2]

[1]Department of Pest Physiology Research, Plant Protection Research Institute, Agriculture Research Center, Egypt
[2]Organic Division, Chemistry Department, Faculty of Science, Zagazig University, Egypt.

Corresponding author: noran.mm@zu.edu.eg, mohamedn490@yahoo.com

ABSTRACT **: The extant study was designed to estimate the basic renotoxicity mechanism prompted by either alone or mixed treatments of zinc oxide nanoparticles (ZnO NPs) and mureer plant (*Senecio glaucus* L.) (SP), and to evaluate the prophylactic activity of gallic acid (GA) against them via examining biochemical, histological, and genetic studies in male rats for 30 days. Forty adult male rats were equally alienated into 8 groups with oral administrated; Control, GA (100 mg/kg), ZnO NPs (150 mg/kg), SP (400 mg/kg), GA+ZnO NPs (100,150 mg/kg), GA+SP (100, 400 mg/kg), ZnO NPs+SP (150, 400 mg/kg), and GA+ZnO NPs+SP (100,150,400mg/kg). Finally, blood and renal tissues were collected. Our results revealed that ZnO NPs and SP induced a significant increase in the concentrations of creatinine (CRE) and urea (URE), with the elevation of total lipid (TL) level compared to the control group, (P<0.001). Also, they triggered congestion in the renal tubules, spreading of inflammatory cells, and degeneration in the lining epithelium. They also induced a strong immunoreactivity of caspase-3 expression. Conversely, GA alleviated and improved renal injury by controlling the presence of inflammatory cells. In conclusion, ZnO NPs and SP could be displayed as nephrotoxic and pro-apoptotic agents; yet, GA exhibits as a renoprotective and an anti-apoptotic agent.**



GRAPHIC ABSTRACT

Keywords: *Zinc oxide nanoparticles, Ethanolic extract of mureer plant, Renal injury, Gallic acid, Caspase-3, Rats.*

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I- INTRODUCTION

Presently, nanotechnology is the most mellowed science all over the world that distinguishes with a very small size of the fabricated products in the range (1–100 nm) **[1]**. Nanoparticles (NPs) enter in medicinal, visual, agricultural, pharmaceutical, and industrial applications. Interestingly, NPs convince many contrary influences in the intracellular tissues due to the inspiration of prolonged cytotoxic pathway, including the production of free radicals, apoptosis mechanism induction, and inflammation reaction stimulation **[2]**.

Amid the renowned well-known NPs, zinc oxide nanoparticles (ZnO NPs) are the prevalent nanoparticles utilized in many profitable purposes, such as aerospace, electronics, optical devices, food packing, sunscreens, building, and computer products. Although all these uses of ZnO NPs spread in the market, they induced many lethal repercussions inside mammalian cells **[3]**. To use friendlier alternative insecticides in the environment, the natural plant could be used due to having many phytochemical compounds triggered negative influences lesser than the old-style chemicals.Among the known indigenous plants, mureer or *Senecio glaucus* subsp. coronopifolius (Maire) C. Alexander L. (SP) exhibits as one member of the Asteraceae family. It spontaneously grows in deserts all over the world that causes many hazardous effects, such as hematoma, diarrhea, and hepatic failure in desert animals **[4]**. In order to identify the cytotoxicity potential of *Senecio* plants, they may be contained many phytochemical constituents, such as alkaloids, phenolic compounds, saponins, coumarins, and flavonoids, which are stored in intracellular tissues causing tissue destruction **[5]**.

From the preceding report, the kidney is the principal organ for blood filtration and waste elimination in the mammalian body, which plays a key role in the transport and clearance of different materials into the body. Additionally, its excretory function depends on the distribution of the prevalent renal blood movement through the glomerular capillaries, which filter the plasma from the metabolic wastes **[6]**. For earlier biochemistry review, creatinine (CRE) and urea (URE) concentrations have been used for the diagnosis of renal dysfunction **[7]**. Notwithstanding the complex cytotoxicological mechanism, apoptosis or programmed cell death is a highly controlled process, which may convince after the stimulation of oxidative stress, ATP depletion, instigation of caspases proteins, and changes in the ratio of apoptosis-related genes **[8].**

There is a rising aspiration for searching for the use of antioxidants or natural supplements in the treatment of toxicity of diverse toxins, such as gallic acid (GA). GA, (3,4,5-trihydroxy benzoic acid), is a polyphenolic compound found in many plants, such as mango, lemon, grape, oak, walnuts, green tea, and other fruits. It has a powerful ability to serve as a protective agent against many damaged impacts **[9]**. Fortunately, GA performs as an anti-mutagenic, anti-inflammatory, cytoprotective, and antidepressant mediator in animal models **[10]**. In order to discern the correlation between the structure of GA and renoprotective activity, GA has an aromatic ring in its organization that can cause scavenging of free radicles because of renal injury **[11]**.

Lastly, the chief purpose of this research was to estimate the renal-toxic impacts induced by ZnO NPs and SP (single or both treatments) via studying biochemical, histopathological, and immunohistochemical examinations. Furthermore, this study targeted to evaluate the promising ameliorative effect of GA against renal toxicity persuaded by ZnO NPs and SP.

II. MATERIALS AND METHODS

Chemicals and reagents

ZnO NPs at the size (<50 nm) (BET), sodium carboxymethyl cellulose salt (Na-CMC), and GA were procured from (Sigma Aldrich Company, St. Louis, Missouri, USA). For plant extraction, 70% of ethanol solvent was obtained from (El-Naser Company-high grade, Egypt). CRE, URE, and total lipid (TL) kits were come from (Diamond and Diagnostic companies, Egypt). Finally, the primary antibody of caspase-3 was gotton from (Santa Cruz Company, USA) and the secondary antibody was bought from (Sigma Company, St. Louis, Missouri, USA).

Preparation and characterization of ZnO NPs

The suspension of ZnO NPs was prepared using 0.5% Na-CMC surfactant sonicated for 20 min in a bath sonicator.

Preparation and extraction of Plant

Entire portions of *mureer* plant were obtained from Cairo-Ismailia Thoroughfare, Egypt. It was known by Dr. Abdel-Halim Abdel-Mogaly, Botanist (Herbarium of Horticultural Research Institute, Agriculture Research Center, Dokki, Giza, Egypt) and recorded in a Uniprot database with Taxon identifier (183639**) [12]**. Leaves, stems, roots, and flowers portions were dried in the air and immersed in 70% ethanol in glass jars for 3 days. At the end of the soaking period, the obtained mixture was filtered with a Whatman paper, concentrated at 60 ˚C in a rotary evaporator (IKA-WERK, RV10, China), and desiccated in an aerated oven at 45 ˚C to advance greenish extract **[13]**.

Experimental Design

The experimental trial was presented following the overall guidelines of Ethics of the Institutional Animal Care and Use Committee (Zagazig University, Egypt). Forty male *Wistar* albino rats (*Rattus norvegicus*), (weighing 180-220 g b.wt, 6-7 weeks age-old), were used after a 1-week period of adaptation. The trial was done in the animal house of the Faculty of Medicine, Zagazig University, Egypt. Rats were allowed a standard pellet diet and tap water *ad libitum* in plastic cages during the trial period. They were kept at an insistent temperature (23±2 ˚C), humidity (60±10%), and a light/dark (12 h:12 h) cycle.

The experimental design was alienated into eight groups that were comprised of five rats in each group: 1) Control group: rats were received 0.5% Na-CMC as a vehicle (5 ml/kg of 0.5% Na-CMC/rat) **[14]**. (2) GA-treated group: rats were received (100 mg/kg of GA) **[15]** appending in 0.5% Na-CMC **[16]**. (3) ZnO NPs-treated group: rats were received (150 mg/kg of ZnO NPs) suspending in 0.5% Na-CMC **[17]**. (4) SP-treated group: rats were received (400 mg/kg of SP). (5) GA+ZnO NPs-treated group: rats were received (GA plus ZnO NPs) at identical used doses. (6) GA+SP-treated group: rats were received (GA plus SP) at similar used doses. (7) ZnO NPs+SP-treated group: rats were received (ZnO NPs plus SP) at alike used doses. (8) GA+ZnO NPs+SP-treated group: rats were received (GA plus ZnO NPs plus SP) at alike used doses. GA complement was administrated before the treatment of other materials. The management of all toxic and ameliorating agents was injected for 30 days via oral administration day by day, which was suspended in 0.5% Na-CMC (w/v).

At the end of 30 days of exposure, animals were sacrificed by cervical dislocation after inhalation with ether. Then, serum was taken after centrifugation at 3,000 rpm for 20 min at 4 ˚C. The supernatant was collected, transferred into vials, and stored at −80 ˚C for subsequent biochemical investigations. The kidney tissue was rapidly removed and kept at 10% neutral buffered formalin for histological as well as immunohistochemical studies.

Estimation of renal function biomarkers

Serum was used to estimate CRE **[18]**, URE **[19]**, and TL **[20]** tests using the calorimetric method.

Histopathological examination

Renal specimens were fixed using 10% neutral buffered formaldehyde. After proper fixation, the specimens were dehydrated in rising grades of ethyl alcohol and embedded in paraffin wax. 5-µm thick sections were cut using a rotatory microtome, deparaffinized, dehydrated, stained with hematoxylin and eosin (H&E.,) staining for studying the general histological structure of the kidney according to **[21]**, and detected under a light microscope.

Immunohistochemistry examination

For the respect of apoptosis-related protein, the paraffin-embedded kidney tissue was censored into a 4-µm section and mounted on positively charged slides for the expression of caspase-3 immunohistochemistry test. The immunohistochemical reaction was done using the peroxidase/anti-peroxidase (PAP) according to the method of **[22]**.

The nonspecific peroxidase reaction was obstructed with methanol containing 0.1% H_2O_2. The segment was also incubated with normal goat serum to dodge nonspecific reaction once the sample was incubated with specific antibodies against caspase-3 (dilution, 1:2000). The tissue sector was then washed with phosphate buffer and incubated with secondary antibodies (dilution, 1:2000). Beforehand, they have washed in phosphate buffer again and lastly incubated with the PAP complex (dilution, 1:200).
The peroxidase reaction was carried out using a solution of 3,3'-diaminobenzidine tetrahydrochloride encompassing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH=7.6). After immunostaining, the kidney section was casually counterstained with (H&E.,) staining noticed under a light microscope.

Statistical analysis

Data were designated as a mean±standard deviation (mean±SD) using one-way ANOVA in the statistical software package SPSS for Windows 20.0 program to make a comparison between the biochemical statistics followed by Tukey's post hoc test for the comparison of multiple groups. The level of significance was prepared at $P<0.05$ **[23].**

III. RESULTS AND DISCUSSION

Our study observed that the noxious effect of ZnO NPs and SP in renal tissue, which persuaded a disruption in the biochemical investigation. Results in a table (1) revealed that either alone or combined treatments of ZnO NPs and SP induced variations in the renal dysfunction indices, such as (creatinine (CRE) (mg/dl), urea (URE) (mg/dl), and total lipids (TL) (mg/dl)). Likewise, Table (1) revealed that ZnO NPs-treated group, SP-treated group and ZnO NPs+SP-treated group) caused a significant increase in serum total lipid (TL) level (1164.43±3.71, 792.64±1.89, 1341.81±7.23) compared to the control group (400.16±2.45), ($P<0.001$).

On the other pointer, GA+ZnO NPs-treated group created a significant reduction relative to ZnO NPs-treated group (845.03±2.89 relative to 1164.43±3.71), GA+SP-treated group provoked a significant drop relative to SP-treated group (552.58±2.18 relative to 792.64±1.89), and GA+ZnO NPs+SP-treated group incited a significant decrease relative to ZnO NPs+SP-treated group (772.04±2.89 relative to 1341.81±7.23), ($P<0.001$).

Stylishly, there was enhancement in the groups, which treated with GA in the values of TL level relative to the alone or combined group of ZnO NPs and SP. Furthermore, the poisonous effect of the combined treatment of ZnO NPs and SP was stronger than the effect of the alone treatment of them. Further, the contrary impact of ZnO NPs-treated group was more than the impact of SP-treated group. Likewise, results revealed that ZnO NPs-treated group, SP-treated group and ZnO NPs+SP-treated group) caused a significant increase in serum urea (URE) concentration (2.70±0.29, 2.56±0.22, 2.86±1.19) compared to the control group (1.43±0.19), ($P<0.001$).

On the other hand, GA+ZnO NPs-treated group made a significant decrease relative to ZnO NPs-treated group (1.83±0.15 relative to 2.70±0.29), GA+SP-treated group incited a significant reduction relative to SP-treated group (1.97±0.90 relative to 2.56±0.22), and GA+ZnO NPs+SP-treated group provoked a significant lessening relative to ZnO NPs+SP-treated group (2.49±0.23 relative to 2.86±1.19), ($P<0.001$).

Tastefully, there was augmentation in the groups, which treated with GA in the values of URE concentration relative to the alone or combined group of ZnO NPs and SP. Furthermore, the lethal effect of the combined treatment of ZnO NPs and SP was stiffer than the effect of the alone treatment of them. Further, the opposing impact of ZnO NPs-treated group was more than the impact of SP-treated group.

Likewise, data revealed that ZnO NPs-treated group, SP-treated group and ZnO NPs+SP-treated group) caused a significant increase in serum creatinine (CRE) concentration (39.86±1.01, 48.54±1.93, 46.50 ±7.83) compared to the control group (34.29±1.59), ($P<0.001$).

On the other hand, GA+ZnO NPs-treated group made a significant decrease relative to ZnO NPs-treated group (25.25±2.24 relative to 39.86±1.01), GA+SP-treated group incited a significant reduction relative to SP-treated group (32.75±3.16 relative to 48.54±1.93), and GA+ZnO NPs+SP-treated group incited a significant declining relative to ZnO NPs+SP-treated group (33.68±3.08 relative to 46.50 ±7.83), ($P<0.001$).

Table 1: Influence of zinc oxide nanoparticles (ZnO NPs), mureer or *Senecio glaucus* L. plant (SP), and gallic acid (GA) on nephrotoxicity biomarkers in serum: creatinine (CRE) (mg/dl), urea (URE) (mg/dl), and total lipid (TL) (mg/dl).

Data were given as mean±SD,(n=5 rats per group). Statistical analysis was made by using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons between groups. Compared with the control group, highly significant: ***($P < 0.001$) and n.s.(P is non-significant). a,b,c,d,e,f,g letters represent the relations between treated groups at $P< 0.05$: [^{a}ZnO NPs relative to SP, ^{b}ZnO NPs+SP relative to ZnO NPs, ^{c}ZnO NPs+SP relative to SP, ^{d}GA+ZnO NPs relative to ZnO NPs, ^{e}GA+SP relative to SP, ^{f}GA+ZnO NPs+SP relative to ZnO NPs+SP, ^{g}GA relative to Control].

Groups	TL (mg/dl)	URE (mg/dl)	CRE (mg/dl)
Control	400.16 ±2.45	1.43±0.19	34.29±1.59
GA	393.79± 3.58 $^{n.s.\ g}$	1.44±0.21$^{n.s.\ g}$	30.53±5.84$^{n.s.\ g}$
ZnO NPs	1164.43±3.71$^{***\ a}$	2.70±0.29$^{***\ a}$	39.86±1.01$^{***\ a}$
SP	792.64 ± 1.89***	2.56±0.22***	48.54±1.93***
GA+ZnO NPs	845.03±2.89$^{***\ d}$	1.83±0.15$^{***\ d}$	25.25±2.24$^{***\ d}$
GA+SP	552.58±2.18$^{***\ e}$	1.97±0.90$^{***\ e}$	32.75±3.16$^{***\ e}$
ZnO NPs +SP	1341.81 ±7.23$^{***\ b,c}$	2.86±1.19$^{***\ b,c}$	46.50 ±7.83$^{***\ b,c}$
GA+ZnO NPs +SP	772.04 ± 2.89$^{***\ f}$	2.49±0.23$^{***\ f}$	33.68±3.08$^{***\ f}$

Sophisticatedly, there was amplification in the groups, which treated with GA in the values of CRE concentration relative to the alone or combined group of ZnO NPs and SP. Furthermore, the lethal effect of the combined treatment of ZnO NPs and SP was harder than the effect of the alone treatment of them. Further, the opposing impact of ZnO NPs-treated group was more than the impact of SP-treated group.

Finally, there were no difference between the control and GA treated group in all these biomarkers. The toxic impact of some plants appears due to the accumulation of saponins as phytochemical compound in renal tissue, which causes hypercholesterolemia, leading to alterations in glomerular size and structure [24]. The accumulation of saponins in renal tissue induces inflammatory reactions causing renal damage [25]. The kidney injury causes a rise of some waste materials in the blood due to failure in the regulation of protein catabolism. Furthermore, it convinces disturbance in the balances of the ion-water, acid-base homeostasis, blood filtration, plasma osmolality, and absorption of calcium through the renal tubules [26]. CRE and BUN measurements are the main biomarkers for the assessment of renal malfunction that are used to evaluate the integrity of renal tissue and the performance for doing its functions [27]. The modification in these parameters persuades due to a reduction of the glomerular filtration, dehydration, and protein metabolism disturbance in the hepatocellular cells [28]. In addition, protein catabolism rises due to the promotion of arginase enzyme production that is responsible for hyperuricemia [29].

Our biochemical analyses were confirmed by the histopathological investigations illustrated in, (Figure 1:a-h). Our results proved that the nephrotoxic influences of ZnO NPs and SP, and the therapeutic influence of GA against them. Firstly, a normal glomerular capsule surrounded by proximal tubules, distal renal tubules, and

interstitial area were observed in the control group, (Figure 1:a). A healthy appearance of the renal tissue was noticed in GA-treated group, (Figure 1:b).

On a hand, there were interstitial dilated congested regions in the renal tubules, spreading of mononuclear inflammatory cell infiltration, and degeneration in the lining epithelial cells of renal tubules in ZnO NPs-treated group, (Figure 1:c). Additionally, interstitial mononuclear inflammatory cell infiltration, tubular cell necrosis, and thickening of the glomerular membrane were perceived in SP-treated group, (Figure 1:d). On the other hand, the addition of GA to both substances induced an improvement in the renal structure and a mild glomerular proliferation in GA+ZnO NPs-treated group, (Figure 1:e). Besides, an appearance of slight glomerular proliferation was seen in GA+SP-treated group, (Figure 1:f). Furthermore, both treatments persuaded an infiltration in the interstitial mononuclear inflammatory cells and severe glomerular proliferation in ZnO NPs+SP-treated group, (Figure 1:g). Likewise, a slight infiltration in the interstitial inflammatory cells was noticed in GA+ZnO NPs+SP-treated group, (Figure 1:h).

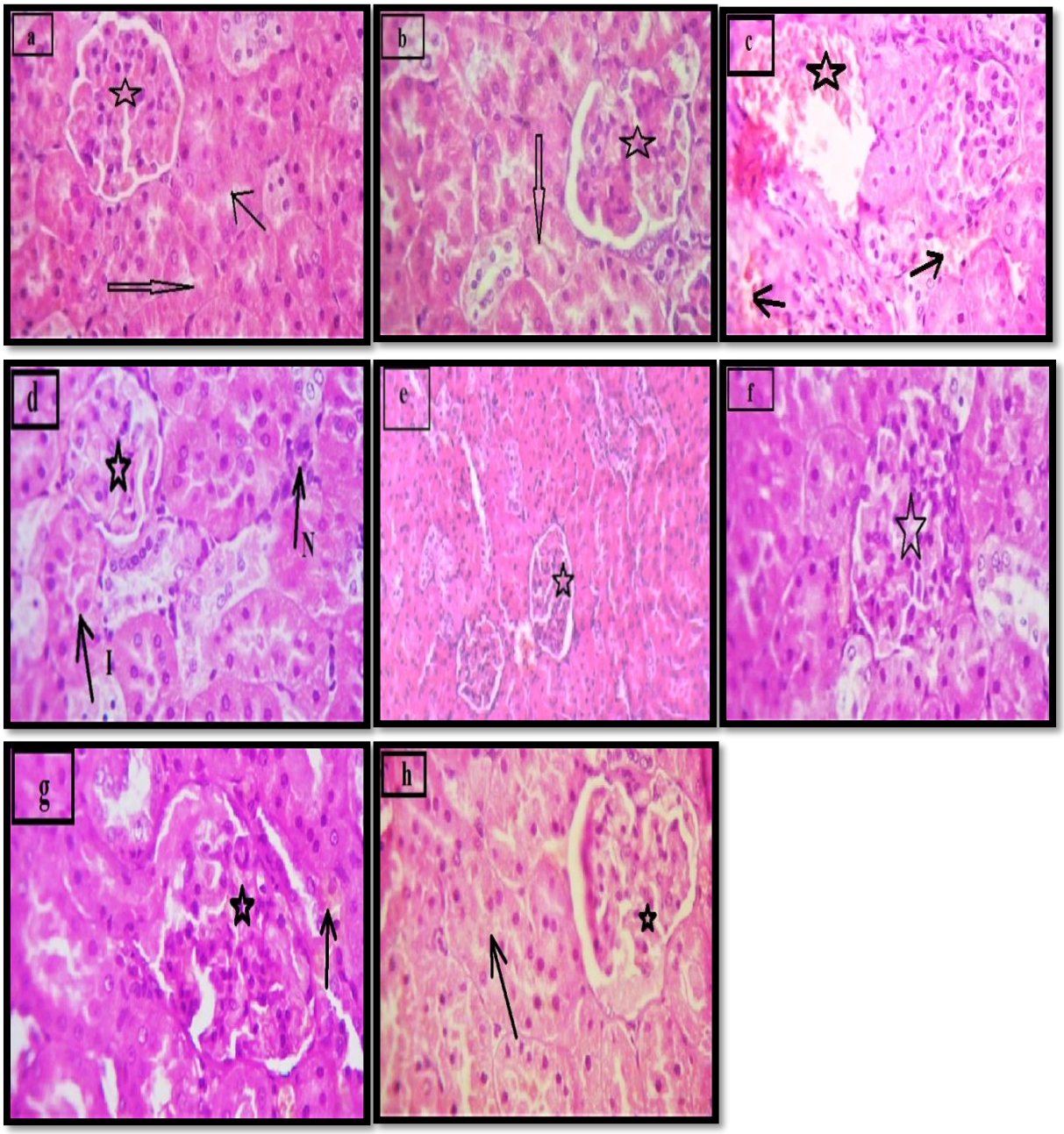

Figure 1(a-h): Photograph of influence of zinc oxide nanoparticles (ZnO NPs), mureer or *Senecio glaucus* L. plant (SP), and gallic acid (GA) of a section of kidney tissue in diverse treated groups: a) Control group presenting a regular appearance of the renal tissue: glomeruli (star), proximal tubules, distal renal tubules (wide arrow), and interstitial area (thin arrow). b) GA-treated group presenting a typical entrance of the renal tissue: glomeruli (star) and renal tubules (wide arrow). c) ZnO NPs-treated group presenting an anomalous entrance of the renal tissue: interstitial dilation of the crowded sectors in the renal tubules (star) and dispersal of mononuclear inflammatory cell infiltration with disintegration in the lining epithelial cells of renal tubules (arrow). d) SP-treated group presenting infiltration in the interstitial mononuclear inflammatory cells (I), tubular cell necrosis (N), and solidifying of the glomerular membrane (star). e) GA+ZnO NPs-treated group presenting glomerular mesangial cell proliferation (star). f) GA+SP-treated group presenting only glomerular mesangial cell proliferation (star). g) ZnO NPs+SP-treated group presenting a gigantic range of infiltration of interstitial mononuclear inflammatory cells (arrow) and spartan glomerular proliferation (star). h) ZnO NPs+SP-treated group presenting standard glomeruli with a slender dwindling in the infiltration district of interstitial inflammatory cells (arrow) and steady glomeruli (star) (H&E.,x400)

The urea cycle involves an arrangement in the mitochondrial amino acid transporters in the liver. However, urea is a highly controlled expression of several enzymes, which controls in the synthesis of nitric oxide, polyamines, proline, glutamate, and glucocorticoids. It also activates an extensive variety of the pro-and anti-inflammatory cytokines and other mediators that are mainly organized by transcriptional factors [30].

Our results were in agreement with, [31] who showed that NPs induced a modification in the renal function test and produced a dilatation in the Bowman's capsule as well as degeneration in renal tubules. Furthermore, our data were in the same line with, [32] who reported that Senecio plant comprised many tributary byproducts, which are responsible for their cytotoxicity effects. To understand the mechanism of renal damage, there are increased fluids in the body, leading to cloudy swelling due to an increase in the sodium, potassium, phosphate levels, and a decrease in the calcium level.

Furthermore, it impaired redox hemostasis causing platelet dysfunction and creating congestion with red blood cells in the interstitial space between the tubules and glomeruli [33]. With regard to an understanding of the reason for the occurrence of hemorrhage in renal cells, they can produce erythropoietin that stimulates the production of red blood cells [34].These disturbances promote trouble in many important enzyme metabolisms, including alkaline phosphatase, gamma-glutamic-trans peptidase, and N-acetyl-β-d-glucosaminidase as result to proteinuria appearance [35].

Our data in (Figure 2:a-h) demonstrated that the pro-apoptotic effect of ZnO NPs and SP and the anti-apoptotic impact of GA against them. Control group and GA-treated group produced a negative immune reaction of caspase-3 expression,(Figure 2:a,b). ZnO NPs-treated group and SP-treated group induced a strong positive immune reaction of caspase-3 expression, (Figure 2:c,d). Conversely, GA+ZnO NPs-treated group and GA+SP-treated group persuaded a mild positive immune response of caspase-3 expression, (Figure2:e,f). Inappropriately, both treatments of ZnO NPs and SP caused a very durable positive immune reaction of caspase-3, (Figure 2:g). Lastly, there was a slight positive immune reaction of caspase-3 expression in GA+ZnO NPs+SP
-treated group, (Figure 2:h).

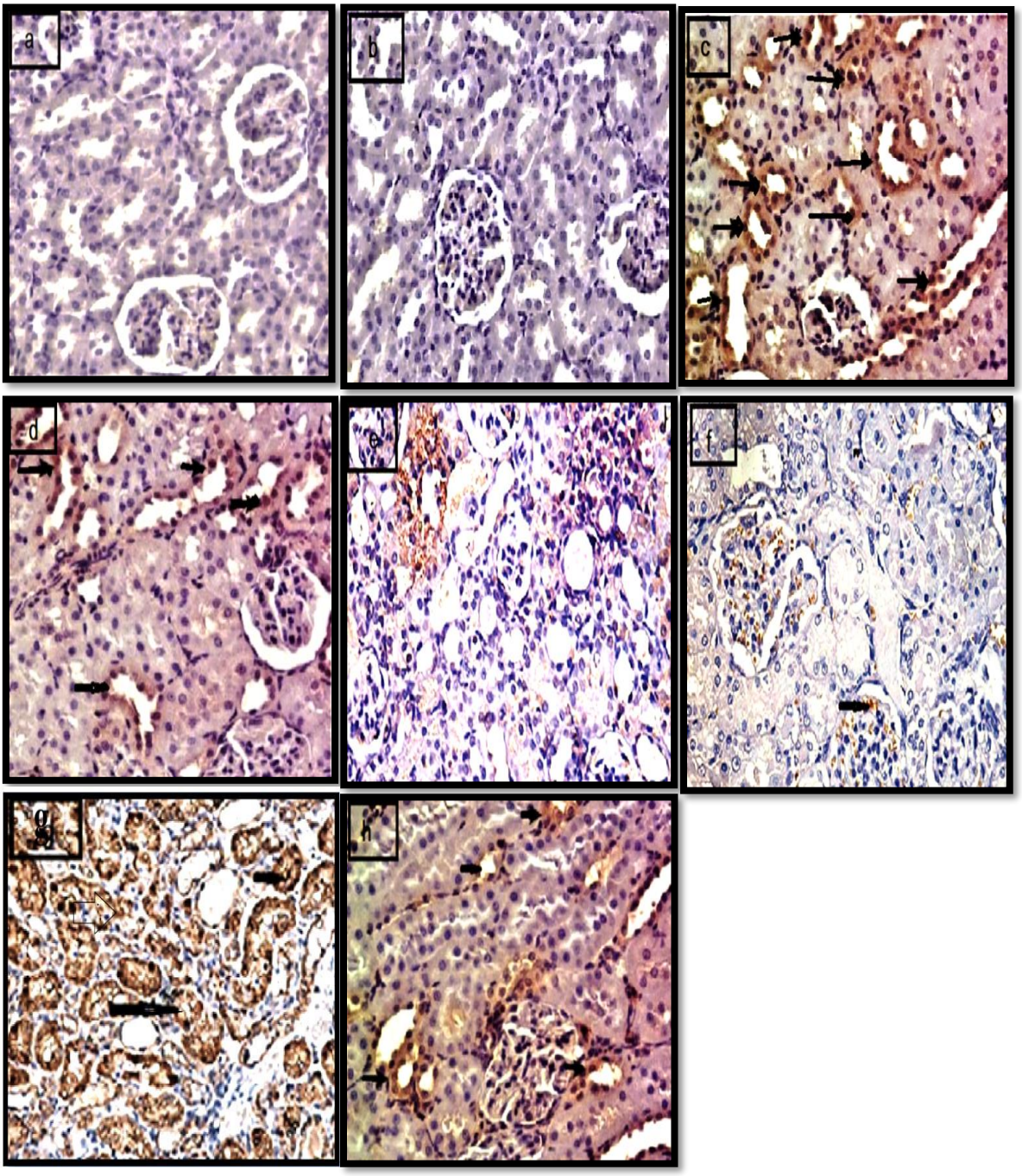

Figure 2(a-h): Photograph of influence of zinc oxide nanoparticles (ZnO NPs), mureer or *Senecio glaucus* L. plant (SP), and gallic acid (GA) on immunohistochemical stain of caspase-3 in renal cells of diverse treated groups. a,b)Control group and GA-treated group presenting a negative immune rejoinder of caspase-3 expression. c,d)ZnO NPs-treated group and SP-treated group presenting a positive immune rejoinder of caspase-3 expression with an advent of dusky brown stain (H&E., x400). e,f) GA+ZnO NPs-treated group and GA+SP-treated group presenting a trivial positive immune rejoinder of caspase-3 expression (H&E.,x200,400). g)ZnONPs+SP-treated group presenting a resilient positive immune rejoinder of caspase-3 expression with scattering of shady brown stain (H&E.,x200). h) GA+ZnO NPs+SP-treated group presenting a judicious positive immune rejoinder of caspase-3 expression (H&E.,x400).

In order to renal damage stimulus, apoptosis caused various physiological adjustments, such as DNA fragmentation, membrane changes, cytoplasmic shrinkage, and cell death. Apoptotic pathways are categorized as being intrinsic (mitochondria-mediated) or extrinsic (receptor-mediated). Predominantly, mitochondria-mediated apoptosis is triggered via the release of apoptotic factors from the mitochondrial intermembrane space into the cytosol, such as cytochrome c [36]. Caspases take part in both pathways called cysteine–aspartic acid protease family, such as caspase-3 protein. It interrelates with caspase-8 and caspase-9 that is liable for morphological variations in the apoptotic cells, such as chromatin condensation, DNA interruption, and cessation of protein [37].

Unfortunately, the proximal tubules are typically susceptible to different damages (obstructive, ischemic, hypoxic, oxidative, and metabolic), resulting from cell death and ultimately in the development of atrophic glomeruli [38]. Our findings were in contract with previous researchers, [39] and [40] who revealed that nanoparticle managements prompted deposition of hyalin-like materials, swelling, dilation in the Bowman capsule, dilation renal tubules, and glomerulus atrophy as a result of induction of CRE as well as URE excretions and DNA damage. From all these biochemical, histopathological, and molecular examinations, our study observed that ZnO NPs and SP managements exhibited as pro-apoptotic and nephrotoxic agents.

Indifference, our study indicated that GA acts as a renoprotective and anti-apoptotic agent against renal toxicity convinced by ZnO NPs and SP. In order to elucidate the modulatory effect of GA against renal damage, it had hydroxyl and carboxyl groups with a benzene ring, which could have overawed the creation of free radicles in injured cells. Thus, it can inhibit inflammatory outbursts, apoptosis, necroptosis, and stimulating glucose uptake [41]. Our biochemical, histological, and molecular analyses about the preserving effect of GA were in the harmony with the preceding study, [42] who reported that GA improved renal dysfunctions, which occurred after diabetes diseases and inhibited the occurrence of DNA damage. Additionally, [43] reported that GA enhanced the histological alternations in renal cells that encouraged afterward diazinon insecticide treatment, such as atrophic glomerular and tubular degeneration due to its alleviation against the oxidative stress.

V–CONCLUSION

To summarize based on the above findings, our results concluded that alone or mixed treatment of ZnO NPs and SP produced renal damage due to immunologically provoking of the overexpression of caspase-3 protein. Thus, they exhibit nephrotoxic and genotoxic agents. In addition, the present study, therefore, provides a biological mechanism supporting the usefulness of GA that has already recovered some of the biochemical, histological, and molecular fluctuations urged by ZnO NPs and SP. Moreover, this study confirmed that GA becomes an optimistic nephron-protective and geno-protective agent that can use as prophylactic activity against kidney damage.

VI- REFERENCES

1. **Mo Y, Zhang Y, Zhang Q.** Evaluation of pulmonary toxicity of nanoparticles by bronchoalveolar lavage. Methods Mol Biol J. 2019; 1894:313-322.
2. **Zhang W, Xiao B, Fang T.** Chemical transformation of silver nanoparticles in aquatic environments: Mechanism, morphology and toxicity. Chemosphere J. 2018; 191:324-334.
3. **Wang C, Lu J, Zhou L, Li J, Xu J, Li W, Zhang L, Zhong X, Wang T.** Effects of long-term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice. PLoS One J. 2016; 11(10):e0164434-e0164450.
4. **Norton J, Majid SA, Allan D, Al Safran M, Böer B, Richer R.** An illustrated checklist of the flora of Qatar. Browndown Publications, Gosport,UK. 2009.

5. **Lizarraga E, Castro F, Fernández F, de Lampasona MP, Catalán CA.** Antioxidant, hemolytic and cytotoxic activities of *Senecio* species used in traditional medicine of Northwestern Argentina. Nat Prod Commun J. 2012; 7(5):607–608.
6. **Du B, Yu M, Zheng J.** Transport and interactions of nanoparticles in the kidneys. Nat Rev Mater J.2018; 3:358–374.
7. **Kataria A, Trasande L, Trachtman H.** The effects of environmental chemicals on renal function. Nat Rev Nephrol J.2015;11(10):610–625.
8. **Mason EF, Rathmell JC.** Cell metabolism: an essential link between cell growth and apoptosis. Biochim Biophys Acta Mol Cell Res J. 2011;1813(4):645–654.
9. **Kosuru RY, Roy A, Das SK, Bera S.** Gallic acid and gallates in human health and disease: Do mitochondria hold the key to success? Mol Nutr Food Res J.2018; 62(1):1700699-1700740.
10. **Jin L, Sun S, Ryu Y, Piao ZH, Liu B, Choi SY, Kim GR, Kim HS, Kee HJ, Jeong MH.** Gallic acid improves cardiac dysfunction and fibrosis in pressure overload-induced heart failure. Sci Rep J.2018; 8(1):9302-9320.
11. **Sroka Z, Cisowski W.** Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol J. 2003; 41(6):753-758.
12. **Boulos L**. Flora of Egypt. Al Hadara Publishing, Cairo, Egypt. 2002; 22 (4):390.
13. **Bahrin N, Muhammad N, Abdullah N, Talip BHA, Jusoh S, Theng SW.** Effect of processing temperature on antioxidant activity of *Ficus carica*. J. Sci. Technol. 2018; 10:99-103.
14. **Dhiyaaldeen SM, Amin ZA, Darvish PH, Mustafa IF, Jamil MM, Rouhollahi E, Abdulla MA.** Protective effects of (1-(4- hydroxyl-phenyl)-3-m-tolyl-propenone chalcone in indomethacin- induced gastric erosive damage in rats. BMC Vet. Res J. 2014; 10:961-975.
15. **Mansouri MT, Farbood Y, Sameri MJ, Sarkaki A, Naghizadeh B, Rafeirad M.** Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. Food Chem J. 2013; 138 (2-3):1028-1033.
16. **Sen S, Asokkumar K, Umamaheswari M, Sivashanmugam AT, Subhadradevi V.** Antiulcerogenic effect of gallic Acid in rats and its effect on oxidant and antioxidant parameters in stomach tissue. Indian J Pharm Sci. 2013; 75(2):149-155.
17. **Srivastav AK, Kumar M, Ansari NG, Jain AK, Shankar J, Arjaria N, Jagdale P, Singh D.** A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in *Wistar* rats: Toxicity study of zinc oxide nanoparticles. Hum Exp Toxicol J. 2016; 35(12):1286-1304.
18. **Hawk PB, Oser BL, Summerson WH.** Practical physiological chemistry. The Blakiston Corporation, 12th Eds.1947; pp.506-509.
19. **Tietz NW.** Clinical guide to laboratory tests. 2nd Eds.W.B. Saunders Company, Philadelphia.1994; pp.1-931.
20. **Zollner N, Kirsch K.** Colorimetric method for determination of total lipids. J Exp Med.1962; 135:545-550.
21. **Bancroft JD, Layton C.** The hematoxylin and eosin. Bancroft's theory and practice of histological techniques, expert consult: online and print, 7th Eds. Elsevier: Amsterdam, Netherlands.2012; pp.173.
22. **Ramos-Vara JA, Kiupel M, Baszler T, Bliven L, Brodersen B, Chelack B, Czub S, Del Piero F, Dial S, Ehrhart EJ, Graham T, Manning L, Paulsen D, Valli VE, West K**. American Association of veterinary laboratory diagnosticians subcommittee on standardization of immunohistochemistry. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. J Vet Diagn Invest. 2008; 20(4):393-413.
23. **IBM Corp SPSS**. IBM SPSS(Statistical package for the social sciences) statistics for windows, version 20.0. Armonk., NY: IBM Corp.; 2011.
24. **Paredes A, Palacios J, Quispe C, Nwokocha CR, Morales G, Kuzmicic J, Cifuentes F.** Hydroalcoholic extract and pure compounds from *Senecio nutans Sch. Bip* (Compositae) induce vasodilation in rat aorta through endothelium-dependent and independent mechanisms. J Ethnopharmacol.2016; 192: 99–107.
25. **Podolak I, Galanty A, Sobolewska D**. Saponins as cytotoxic agents:A review. Phytochem Rev J. 2010;9(3):425–474.
26. **Herzlinger D, Hurtado R.** Patterning the renal vascular bed. Semin Cell Dev Biol J. 2014;36:50–56.
27. **Rao R, Bhalla V, Pastor-Soler NM.** Intercalated cells of the kidney collecting duct in kidney physiology. Semin Nephrol J. 2019; 39(4):353-367.

28. **Uchino S, Bellomo R, Goldsmith D.** The meaning of the blood urea nitrogen/creatinine ratio in acute kidney injury. Clin Kidney J. 2012; 5(2):187-191.
29. **Ruilope LM, Garcia-Puig J.** Hyperuricemia and renal function. Curr Hypertens Rep J. 2001; 3(3):197-202.
30. **Morris SM Jr.** Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr J.2002; 22: 87-105.
31. **Fartkhooni FM, Noori A, Mohammadi A.** Effects of titanium dioxide nanoparticles toxicity on the kidney of male rats. Int J Life Sci. 2016; 10(1):65-69.
32. **Parra C, Soto E, León G, Salas CO, Heinrich M, Echiburú-Chau C.** Nutritional composition, antioxidant activity and isolation of scopoletin from *Senecio nutans*: support of ancestral and new uses. Nat Prod Res J.2018; 32(6):719-722.
33. **Weiner ID, Mitch WE, Sands JM.** Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol. 2015; 10(8):1444–1458.
34. **Ogobuiro I, Tuma F.** Physiology, renal. In:StatPearls.[Treasure Island (FL): StatPearls Publishing; 2020] Available from: https://www.ncbi.nlm.nih.gov/books/ NBK538339/.
35. **Wang L, Li J, Li J, Liu Z.** Effects of lead and/or cadmium on the oxidative damage of rat kidney cortex mitochondria. Biol Trace Elem Res J. 2010; 137(1):69-78.
36. **Fulda S, Debatin KM.** Resveratrol modulation of signal transduction in apoptosis and cell survival: a mini-review. Cancer Detect Prev J.2006; 30(3):217-223.
37. **D'Amelio M, Cavallucci V, Cecconi F**. Neuronal caspase-3 signaling: not only cell death. Cell Death Differ J. 2010; 17:1104–1114.
38. **Chevalier RL**. The proximal tubule is the primary target of injury and progression of kidney disease: role of the glomerulotubular junction. Am J Physiol Renal Physiol. 2016; 311(1): F145–F161.
39. **AL-Doaiss AA, Ali D, Ali BA, Jararr BM**. Renal histological alterations induced by acute exposure of titanium dioxide nanoparticles. Int J Morphol.2019; 37(3): 1049-1057.
40. **Yousef MI, Mutar TF, Kamel MAE**. Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicol Rep J. 2020; 6: 336–346.
41. **Akomolafe SF, Akinyemi AJ, Anadozie SO.** Phenolic acids (gallic and tannic acids) modulate antioxidant status and cisplatin induced nephrotoxicity in rats. Int Sch Res Notices J. 2014; 2014: 984709-984717.
42. **Ahad A, Ahsan H, Mujeeb M, Siddiqui WA.** Gallic acid ameliorates renal functions by inhibiting the activation of p38 MAPK in experimentally induced type 2 diabetic rats and cultured rat proximal tubular epithelial cells. Chem Biol Interact J. 2015; 240: 292–303.
43. **Ajibade TO, Oyagbemi AA, Omobowale TO, Asenuga ER, Afolabi JM, Adedapo AA.** Mitigation of diazinon-induced cardiovascular and renal dysfunction by gallic acid. Interdiscip Toxicol J.2016; 9(2):66–77.