International Journal of Scientific and Research Publications, Volume 5, Issue 6, June 2015
ISSN 2250-3153

The renoprotective effect of gum arabic in gamma-irradiated and cisplatin treated rats

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Abstract- Nephrotoxicity is a dose-dependent side effect of cisplatin and γ-radiation that limits their clinical usage in the field of cancer treatments. The present study was designed to evaluate the protective effect of gum Arabic (GA) on nephrotoxicity induced in rats by cisplatin and γ-radiation each alone or combined together. Biochemical investigation of kidney function tests, oxidative stress markers, tumor necrosis factor-alpha (TNF-α) and myeloperoxidase (MPO) was carried out. Renal trace elements contents (Cu, Zn, Fe, Ca, Mg, Mn, Se and Pt), histopathological examination of kidney tissues and immunohistochemical determination of inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) were also performed. Results obtained revealed significant elevation in serum urea and creatinine; propagation in lipid peroxidation (MDA); elevation in nitric oxide (NO) concentration, with subsequent elevation of proinflammatory cytokines as TNF-α as well as increased activities of iNOS, eNOS and myeloperoxidase (MPO), parallel to decline in reduced glutathione (GSH) content. Alterations in renal trace elements contents were detected due to treatment with cisplatin and/or γ-radiation. Moreover, histopathological examination of kidney tissue reflected marked injury. Supplemention with GA significantly ameliorated these parameters, indicating its renoprotective and antioxidant properties. In conclusion, pretreatment with GA offers protection against cisplatin and γ - radiation-induced renal cellular damage.

Index Terms- cisplatin, γ-radiation, gum Arabic, kidney function, oxidative stress.

I. INTRODUCTION

The new generation of platinum-based cytotoxic agents, cisplatin (cis-diamminedichloroplatinum II, CDDP) remains a highly effective and widely used anti-neoplastic drug against various solid tumors, including endometrial, testicular, ovarian, breast, bladder, head, neck and lung cancers. Molecular mechanism of chemotherapy-induced toxicity revealed that cisplatin-induced nephrotoxicity occurs mainly through accumulation of cisplatin in renal tubular cells [1]. Cisplatin-induced renal damage is associated with increased renal vascular resistance and histological damage to proximal tubular cells. The alterations induced by cisplatin in the kidney functions are characterized by sign of injury, such as increase in creatinine and urea, decrease in body weight, decrease in GSH content and increase of lipid peroxidation products [2].

Ionizing radiation (IR) is an important environmental risk factor for various cancers and is also a major therapeutic agent for cancer treatment. Exposure of mammalian cells to IR induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage, as well as DNA-DNA and DNA-protein cross-links. During radiotherapy, IR particles interact with biological systems to induce excessive oxygen free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, thereby leading to significant cellular damage. ROS has negative impact on the antioxidant defense mechanisms by reducing the intracellular concentration of GSH, and decreasing the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [3]. One of the indices of oxidative damage is malondialdehyde (MDA) formation as an end product of lipid peroxidation. Lipid peroxidation products such as MDA form adducts with cellular DNA [4] and thus, scavenging free radicals and inhibiting lipid peroxidation are likely key target activities for developing successful radioprotection strategies.

Gum Arabic (GA) is a dried gummy exudate from the stems and branches of *Acacia senegal* (*Leguminosae*). It contains calcium, magnesium, and potassium salts of the polysaccharide GA acid. GA has been used in Arab's folk medicine to reduce both the frequency and the need for hemodialysis in chronic renal failure patients [6]. Furthermore, GA has been shown to reduce urinary nitrogen excretion by increasing urea disposal in the cecum and lowering serum urea concentration in rat and human [5]. Additionally, it was reported that treatment with GA significantly prevented gentamicin-induced lipid peroxidation in the kidney tissue and protected against gentamicin-induced changes in renal function and histological changes [6]. GA also possesses a powerful antioxidant effect through scavenging of superoxide anions [7].

The present work aimed to threw more light on the potentiality of GA as a powerful antioxidant in reducing γ-radiation and cisplatin-induced renal cellular damage.

II. MATERIALS AND METHODS

Animals

Adult male Wistar albino rats, weighing 120-150 g, were obtained from the institute of ophthalmology (Giza, Egypt). The animals were kept under suitable laboratory conditions throughout the period of investigation. They were allowed free

access to food consisting of standard pellets obtained from El-Nasr chemical company (Cairo, Egypt) and water *ad-libitum*. The study was carried out according to the approval of Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University and in accordance with the guidelines set by the EEC regulations (revised directive 86/609/EEC) at the National Center for Radiation Research and Technology.

Chemicals

Gum Arabic powder was purchased from El-gomhouria company (Cairo, Egypt), freshly suspended in distilled water and administered p.o. daily for two weeks in dose of 7.5% g/kg [2]. Cisplatin was obtained from Hospira (UK, Australia), injected intraperitoneally in a single nephrotoxic dose of 7.5 mg/kg [2]. All other chemicals and used solvents were of the highest purity and analytical grade.

Irradiation

Whole-body γ-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT; Cairo, Egypt) using an AECL Ci-137 Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6.5 Gy of gamma radiation at dose rate of 0.758 rad/ sec calculated according to the dosimeter department in the NCRRT.

Experimental design

Adult male Wistar rats were classified into eight groups (n=8). Group I received distilled H_2O p.o. daily for two weeks and served as normal group. Group II received GA (7.5% g/kg; p.o.) daily for two weeks. Group III received cisplatin (7.5 mg/kg; i.p.) as a single dose at the 10^{th} day. Group IV received GA daily for ten days followed by a single i.p. injection of cisplatin then GA daily for another 4 days. Group V was irradiated with a single dose of γ-radiation (6.5 Gy). Group VI received GA daily for one week then irradiated at 7^{th} day followed by GA daily for another week. Group VII were irradiated (6.5 Gy) then subjected to single i.p. injection of cisplatin after 2 days of irradiation. Group VIII received GA daily for two weeks, subjected to irradiation treatment at the 7^{th} day and cisplatin injection at the 10^{th} day. Twenty-four hours after the last dose of specific treatments, blood samples were withdrawn via the retro-orbital venous plexus using heparinized capillary tubes [8]. Serum was separated by centrifugation at 3000 rpm for 15 min and kept frozen. Animals were anesthetized with ether and were sacrificed. Kidneys were quickly excised, washed with distilled water, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA) to prepare 10% homogenate in saline. Samples were then kept frozen until analysis of biochemical parameters. Other kidney samples were fixed in 10% formalin for histopathological and immunohistochemical study.

Estimation of serum creatinine, urea, uric acid and glucose levels

Serum creatinine [9], urea [10], uric acid [11] and glucose [12] were measured colorimetrically using a test reagent kits.

Estimation of renal lipid peroxide, reduced glutathione and nitric oxide contents

Lipid peroxidation was determined by estimation of thiobarbituric acid reactive substances (TBARS) measured as MDA [13]. On the other hand reduced glutathione (GSH) was determined according to Ellman's method [14]. Total nitric oxide (NO) was determined spectrophotometrically [15] based on the enzymatic conversion of nitrate to nitrite by nitrate reductase.

Estimation of renal myeloperoxidase activity and tumer necrosis factor-α content

Myeloperoxidase activity was measured [16] in tissues. Meanwhile, TNF-α was determined using ELISA (Quantikin R & D system, USA) according to the manufacturer' instructions [17].

Histopathological study

For histopathological examination kidney paraffin sections (5 µm thick) were stained with hematoxylin and eosin [18] and examined under light microscope.

Immunohistopathological study

For immunohistochemical determination of inducible nitric oxide (iNOS) and endothelial (eNOS), sections 4 µm thick were cut from paraffin-embedded rat kidney tissues. The samples were first exposed to 60° C overnight and then kept in xylene for 30 min and rehydrated using a series of ethanol solutions for 2 min each, thereafter the sections were washed with distilled water and phosphate buffer saline (PBS) for 10 min. Then they were kept in 2% trypsin in Tris buffer at 37° C for 15 min and washed with PBS three times for 5 min. The sections were incubated in 3% hydrogen peroxidase for 15 min to inhibit endogenous peroxidase activity. Then the tissues were washed with PBS three times for 5 min each and stained with primary antibodies; polyclonal anti-iNOS (clone RB-9242-R7, ready to use; Thermo Fisher Scientific Anatomical Pathology, Cheshire, United Kingdom) and monoclonal anti-eNOS (clone RB-9279; Thermo Fisher Scientific Anatomical Pathology, Cheshire, United Kingdom) for 18 h. After washing the secondary antibody (biotinylated goat IgG anti-rabbit/mouse IgG, Histostain-plus bulk kit Zymed 85- 9043, California, USA) was applied for 30 min. followed by three washes in PBS. The streptavidinperoxidase complex was added for 30 min and washed in PBS three times. Sections were then stained with diaminobenzidine (DAB, Dako) to detect immunoreactivity and then counter-stained with Mayer's hematoxylin. The presence of a brown precipitate indicated positive findings for the primary antibodies. The negative controls received the same treatment with rabbit IgG or mouse IgG instead of primary antibodies [19]. They were covered with mounting medium and observed under an Olympus BX-40 light microscope.

Trace metals analysis

Iron (Fe), cupper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), manganese (Mn), selenium (Se) and platinum (Pt) concentrations were measured in kidney tissue as well as in GA solution. The digestion process used Milestone MLS-1200 Mega and High Performance Microwave Digestor Unit (Italy). Of each organ 0.5-1 g was put in special vessels with 6 ml nitric acid and

1 ml hydrogen peroxide. After complete digestion, samples were diluted to suitable levels for metals analysis by Thermo Scientific ICE 3000 series Atomic Absorption Spectrophotometer (AAS) (England) [20].

Statistical analysis

Values were calculated as mean ± standard error (S.E) of the mean. Comparison between different groups was carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test using Instat software, version 3 **(Graph pad Software, Inc., San Diego, USA)**. The *p* value was set at < 0.05. The figures were drawn using instant software program (Microsoft Office Excel 2003).

III. RESULTS AND DISCUSSION

Effect of GA on serum creatinine, urea, uric acid and glucose levels in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Daily GA administration in a dose of 7.5% g/kg to normal animals for two weeks did not significantly alter kidney functions tests or glucose level. Administration of cisplatin, exposure to γ-radiation or both of them significantly increased rat serum levels of creatinine to 467%, 250% and 489% and urea to 298%, 218% and 314%, respectively as compared to the normal group, while, serum uric acid level did not show any changes compared to normal value. Rats treated with cisplatin, exposed to γ-radiation or both when supplemented with GA significantly reduced levels of serum creatinine to 50%, 29% and 13% and urea to 76%, 62% and 67%, respectively as compared to their respective controls ***(Table 1)***.

In the current study administration of cisplatin, exposure to γ-radiation or both of them significantly increased serum levels of creatinine and urea compared to the normal group.

It has been shown that cisplatin caused a significant increase in blood urea nitrogen and creatinine levels when compared to control group [21]. The increase in blood creatinine and urea has been reported after exposure to radiation and secondary to renal damage. In addition, the elevation in urea may be attributed to an increase in nitrogen retention or excessive protein breakdown [22]. The results obtained are similar to those recorded in another study [23] and add further to the fact that cisplatin at a dose of 7 mg/kg severely impairs renal function in rats. Uptake of cisplatin is mainly through the organic transporter pathway. The kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. Accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity. Cisplatin is accumulated by peritubular uptake in both the proximal and distal nephrons [24].

Treatment with GA was associated with 24 h increased creatinine clearance in healthy mice [25]. GA is fermented by intestinal bacteria leading to formation of various degradation products, such as short-chain fatty acids [26, 27]. Serum butyrate concentrations were increased following treatment with GA in healthy subjects and this may have a role in the claimed salutatory effect on creatinine clearance and glomerular filtration rate. GA was given at an oral dose of 50 g/day for 3 months, with or without supplementing the diet with ferrous sulfate (200 mg/day) and folic acid (5 mg/day) [28]. Serum creatinine, urea, phosphate and uric acid concentrations were reported to be significantly reduced by GA, while the treatment significantly increased that of serum calcium.

Administration of cisplatin, exposure to γ-radiation or both of them significantly increased rat serum level of glucose to 99%, 114% and 122%, respectively as compared to the normal group. GA also normalized the hyperglycemia condition to 84.80%, 62.33% and 92.49% respectively as compared to their respective controls ***(Table 1)***.

A significant hypoglycemic effect was exerted by GA in normal but not in alloxan diabetic rabbits. GA may act as hypoglycemic agent by initiating the release of insulin from pancreatic beta cells of normal rabbits [29]. Mixtures of different types of gum have been shown to inhibit glucose movement in vitro, and lower postprandial blood glucose and plasma insulin in human subjects when incorporated in a drink containing 50 g glucose [30, 31].

Effect of GA on renal MDA, GSH and NO contents in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Administration of cisplatin, exposure to γ-radiation or both of them significantly increased contents of MDA to 152%, 136% and 164% and NO to 166%, 156% and 174%, respectively as compared to the normal group. Meanwhile, renal GSH contents were significantly decreased to 53%, 53% and 55%, respectively as compared to the normal group. Daily treatments of rats with GA significantly reduced MDA contents to 56%, 76% and 55% and NO to 76%, 72% and 61%, respectively as compared with their respective controls. This was coupled by enhanced renal GSH contents to 176%, 188% and 145%, respectively as compared with respective control values ***(Table 1)***.

Administration of cisplatin, exposure to γ-radiation or both of them significantly increased renal lipid peroxidation and decreased GSH content compared to the normal group in the present study. Cisplatin-induced mitochondrial ROS generation triggered inflammatory response, cell death and kidney dysfunction/ nephropathy. These findings point to the presence of oxidative stress and are in accordance with data reported in the literature [32].

Cisplatin has a synergistic cytotoxic action with radiation and other chemotherapeutic agents [33]. Cisplatin administration induced overproduction of ROS such as hydrogen peroxide and hydroxyl radicals, which abstract a hydrogen atom from polyunsaturated fatty acids and depletes the cellular antioxidant capacity [34]. Overproduction of ROS is a harmful process that can be an important mediator of damage to cell structures, including lipids, membranes, proteins, and DNA. Most cell damage caused by IR is also mediated by ROS generated from the interaction between radiation and water molecules in cells [35, 36].

The mechanism of cisplatin-induced nephrotoxicity is still not fully clear. *In vitro* and *in vivo* studies provide strong evidence that implicates oxidative stress as a contributor of cisplatin-induced nephrotoxicity [37]. Cisplatin was found to generate superoxide and hydroxyl radicals and to stimulate renal lipid peroxidation. As a result, an imbalance between generation of oxygen-derived radicals and endogenous enzymatic and non enzymatic antioxidants will occur leading to oxidative damage of cell components [37]. The nephrotoxicity of cisplatin is the result

of the binding of cisplatin to GSH and the subsequent metabolism of the cisplatin-GSH complex (a platinum- GSH conjugate) via a γ-glutamyl transpeptidase (GGT)-dependent pathway in the proximal tubules [38]. Additionally, increased kidney content of platinum caused GSH depletion after cisplatin administration and induced its nephrotoxicity. Therapeutic effects of cisplatin based on its interaction with DNA in the cell, prevent proliferation and induce apoptosis in tumor cells. Renal insufficiency is the major and most severe form of toxicity associated with use of cisplatin as a chemotherapeutic agent.

In the current work, administration of cisplatin, exposure to γ-radiation or both of them significantly increased NO contents coupled with increased activity of iNOS and eNOS in kidney tissues compared to the normal group. The renal content of peroxynitrite and NO is increased in cisplatin treated rats [24]. Peroxynitrite causes changes in protein structure and function, lipid peroxidation, chemical cleavage of DNA, and reduction in cellular defenses by oxidation of thiol pools [24].

In addition, it has been shown that cisplatin increased thiobarbituric acid reactive substances (TBARS) and NO which are known among the pathogenic intermediates triggering DNA damage [39]. Cisplatin triggers cellular responses involving multiple pathways, including DNA repair, transcription inhibition, cell cycle arrest, cellular transport system impairment, ATPase activity reduction and mitochondrial damage [40]. Cisplatin administration caused remarkable deterioration in antioxidant defense as evidenced by decreased glutathione-S-transferase (GST), glutathione peroxidase (GP_X), catalase (CAT) and superoxide dismutase (SOD) in kidney of control, and treated animals and increased TBARS, NO and xanthine oxidase in renal tissues. The oxidative stress mainly results from formation of cisplatin-GSH conjugation. The conjugation contributes to GSH depletion and alteration of redox state in kidney and consequently leads to an increase in generation of superoxide and other oxygen radicals [39].

Gum acacia, which was useful for protection against gentamicin-induced nephrotoxicity [41], offers some protection in renal MDA induced by cisplatin indicating a possible antioxidant effect. Treatment with GA reduced the cisplatin-induced renal and mitochondrial oxidative stress, restored mitochondrial respiratory enzyme activities and attenuated expressions of apoptosis and inflammation related proteins, thus forming the molecular basis for protective mechanism of GA against cisplatin-induced nephrotoxicity [42].

Effect of GA on renal MPO activity and TNF-α content in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Kidney MPO activity and TNF-α content of the normal animals were 4.08±0.12 U/g and 30.08±1.72 pg/g tissue, respectively. Administration of cisplatin, γ-radiation and both of them significantly increased kidney MPO activities to 182%, 172% and 220%, and of TNF-α contents to 331%, 315% and 375%, respectively as compared to the normal group. Daily treatments of rats with GA significantly reduced MPO activities in kidney to 76%, 67% and 63%, and contents of TNF-α in kidney to 64%, 60% and 68%, respectively as compared with their respective control values ***Figure (1& 2)***.

Cisplatin, exposure to γ-radiation or both of them significantly enhanced renal TNF-α content and MPO activity as a major neutrophil protein in kidney compared to normal group. MPO is an essential enzyme for normal neutrophil function and it's a heme enzyme that uses the superoxide and hydrogen peroxide generated by the neutrophil oxidative burst to produce hypochlorous acid and other reactive oxidants, and when neutrophils are stimulated by various stimulants, MPO increases like other cellular tissue-damaging substances [43]. MPO is considered as an important component of the neutrophils antimicrobial defense mechanism [44]. TNF-α induces apoptosis that produces ROS and coordinates the activation of large network of chemokines and cytokines in kidney [34, 45]. The *in vivo* mechanisms of cisplatin nephrotoxicity are oxidative stress, apoptosis, inflammation, and fibrogenesis. ROS are produced via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells. Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production [24]. Cisplatin increased kidney levels of TNF-α, TNF-α mRNA, interleukin 6 mRNA and tumor suppressor protein p53 mRNA in rats treated with cisplatin [39]. Also, the present results of histology showed obvious tissue damage in kidney, including vacuolization, severe necrosis, degenerative changes in lining epithelium of renal tubules and desquamation of degenerated cells present in the lumen of the tubules.

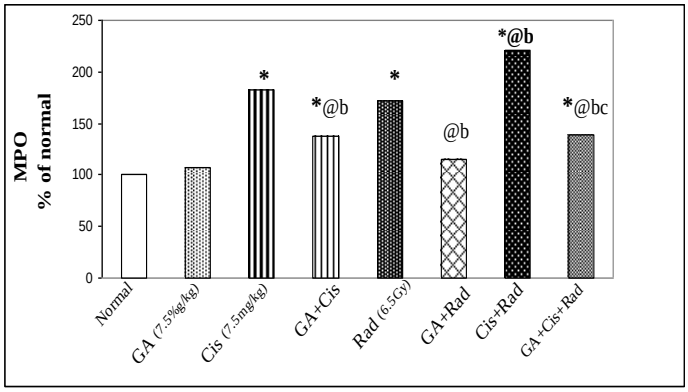

Figure (1): Effect of gum Arabic (GA) on renal myeloperoxidase (MPO) activity in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Each value represents mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

*Significantly different from normal group at $p < 0.05$.
@Significantly different from cisplatin group at $p < 0.05$.
bSignificantly different from γ-irradiated group at $p < 0.05$.
cSignificantly different from cisplatin and γ-irradiated group at $p < 0.05$.

Table (1): Effect of gum Arabic (GA) on serum levels of creatinine, urea, uric acid and glucose as well as renal contents of malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Parameters / *Groups*	**Creatinine** (mg/dl)	**Urea** (mg/dl)	**Uric acid** (mg/dl)	**Glucose** (mg/dl)	**MDA** (nmol/g) tissue	**GSH** (mg/g) tissue	**NO** (µmol/g) tissue
Normal **(**distilled H_2O**)**	0.64 ± 0.05	8.19 ± 0.44	2.07 ± 0.13	102.60 ± 5.25	161.70 ± 14.11	263.40 ± 19.50	159.00 ± 4.09
GA (7.5%g/kg; p.o.)	0.75 ± 0.01	9.67 ± 0.69	2.06 ± 0.20	108.20 ± 2.72	143.11 ± 11.38	250.00 ± 10.32	167.50 ± 2.47
Cisplatin (7.5mg/kg;i.p.)	* 2.99 ± 0.20	* 24.37 ± 1.22	2.22 ± 0.11	101.90 ± 8.55	* 245.30 ± 7.87	* 138.20 ± 9.33	* 263.20 ± 3.71
GA + Cisplatin	*@ 1.49 ± 0.09	*@ 18.44 ± 0.92	1.89 ± 0.08	b 86.42 ± 1.74	@b 136.60 ± 6.73	@b 243.70 ± 12.64	*@b 200.20 ± 1.50
γ-radiation (6.5 Gy)	*@ 1.60 ± 0.02	*@ 17.81 ± 0.57	2.11 ± 0.08	117.10 ± 5.97	* 219.90 ± 15.07	* 140.00 ± 5.55	*@ 247.30 ± 0.86
GA + γ-radiation	@b 0.46 ± 0.01	@b 10.96 ± 0.79	2.37 ± 0.13	*@b 73.00 ± 2.36	@b 167.00 ± 6.21	@b 263.60 ± 12.83	*@b 177.20 ± 0.51
Cisplatin+ γ-radiation	*b 3.13 ± 0.18	*b 25.68 ± 0.54	2.26 ± 0.14	*@ 125.20 ± 2.39	*b 265.30 ± 7.87	* 143.90 ± 5.72	*@b 277.10 ± 1.18
GA + Cisplatin + γ-radiation	@bc 0.41 ± 0.01	*@c 17.10 ± 0.99	c 1.67 ± 0.06	115.80 ± 2.17	*@bc 146.90 ± 2.63	*bc 208.20 ± 14.46	*@bc 170.20 ± 1.81

Each value represents mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

* Significantly different from normal group at $p < 0.05$.

[@]Significantly different from cisplatin group at $p < 0.05$.

[b]Significantly different from γ-irradiated group at $p < 0.05$.

[c]Significantly different from cisplatin and γ-irradiated group at $p < 0.05$.

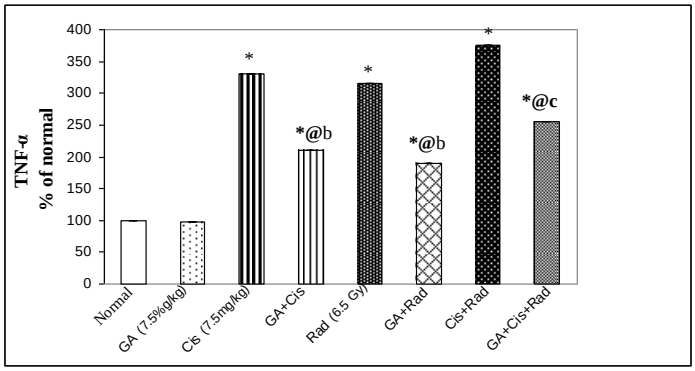

Figure (2): Effect of gum Arabic (GA) on renal tumor necrosis factor-alpha (TNF-α) content in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Each value represents mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.
*Significantly different from normal group at $p < 0.05$.
[@]Significantly different from cisplatin group at $p < 0.05$.
[b]Significantly different from γ-irradiated group at $p < 0.05$.
[c]Significantly different from cisplatin and γ-irradiated group at $p < 0.05$.

Histopathological study

Subjection of rats to cisplatin or γ-irradiation either alone or combined resulted in marked histopathological alterations shown by the appearance of damaged glomeruli, profligacy lesion in the convoluted tubules, mesangial disorganization inside the Bowman's capsule and inflammable obstructed appearance of cortical convoluted tubules ***(Figures 3 A, C, D & E)***. GA administration retarded the previous histopathological alterations where the normal appearance of renal Bowman's capsule, glomeruli, proximal tubules and distal convoluted tubules was shown ***(Figures 3 B, F, G & H)***.

Effect of GA on renal eNOS and iNOS immunohistochemical expressions in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Administration of cisplatin, exposure to γ-radiation and their combination increased expression in iNOS and eNOS staining in renal tissues as compared to the normal group ***(Figures 4 &5).*** Daily treatments of rats with GA for two weeks reduced iNOS and eNOS expression as compared with their respective controls ***(Figures 4& 5)***

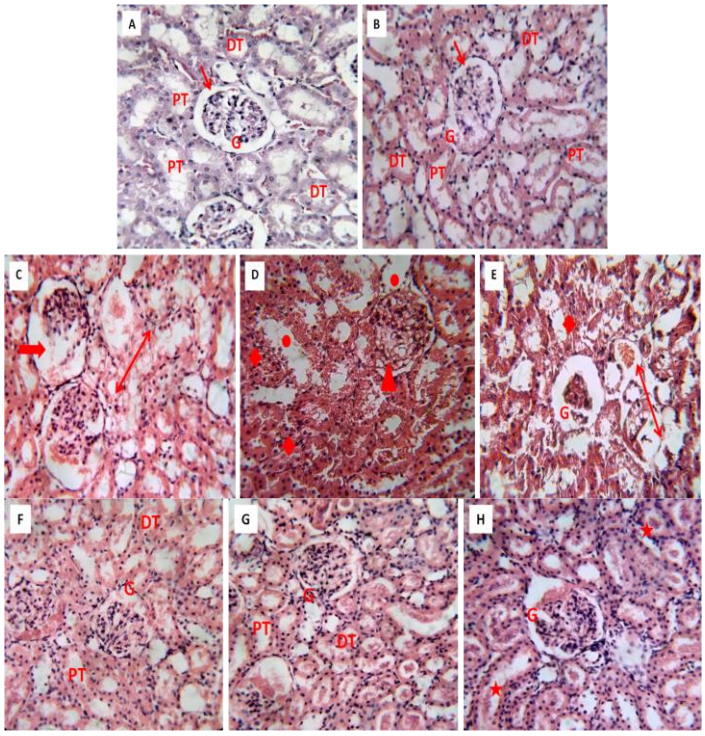

Figure (3): Photomicrographs of kidney sections in rat.
A. Normal control rat kidney showing cortical part of a control rat. The renal Bowman's capsule (↓) and glomeruli (G) show normal structure and the proximal tubules (PT) lined with typical thick cubic epithelium and distal (DT) convoluted tubules lined with the relatively low simple cubic epithelium. **B.** Arabic gum control rat showing the normal appearance of renal Bowman's capsule (↓), glomeruli (G), proximal tubules (PT) and distal (DT) convoluted tubules **C.** Cisplatin control rat showing the appearance of damaged glomeruli (⬆) and profliagcy lesion (↔) in the convoluted tubules. Meanwhile, other convoluted tubules containing marginal chromatin and debris of rupture cells. **D.** γ-Irradiated control rat showing damaged glomeruli, mesangial disoragnization and amorphous eosinophilic content (▲) inside the Bowman's capsule, inflammable obstructed appearance (◆) of cortical convoluted tubules in addition to the presence profliagcy lesion in others (●). **E.** Irradiated rat treated with cisplatin showing the presence of atrophied glomeruli (G), some obstruction (◆) profliagcy lesion (↕) in other convoluted tubules. **F.** Cisplatin rat treated with AG showing the normal appearance of renal Bowman's capsule, glomeruli (G), proximal tubules (PT) and distal (DT) convoluted tubules. **G.** Irradiated rat treated with AG showing the the normal appearance of renal Bowman's capsule, glomeruli (G), proximal tubules (PT) and distal (DT) convoluted tubules. **H.** Irradiated rat treated with cisplatin and AG showing the normal appearance of renal Bowman's capsule and glomerulus (G), in addition some convoluted tubules show some blood coagulation (★). (H&E X400)

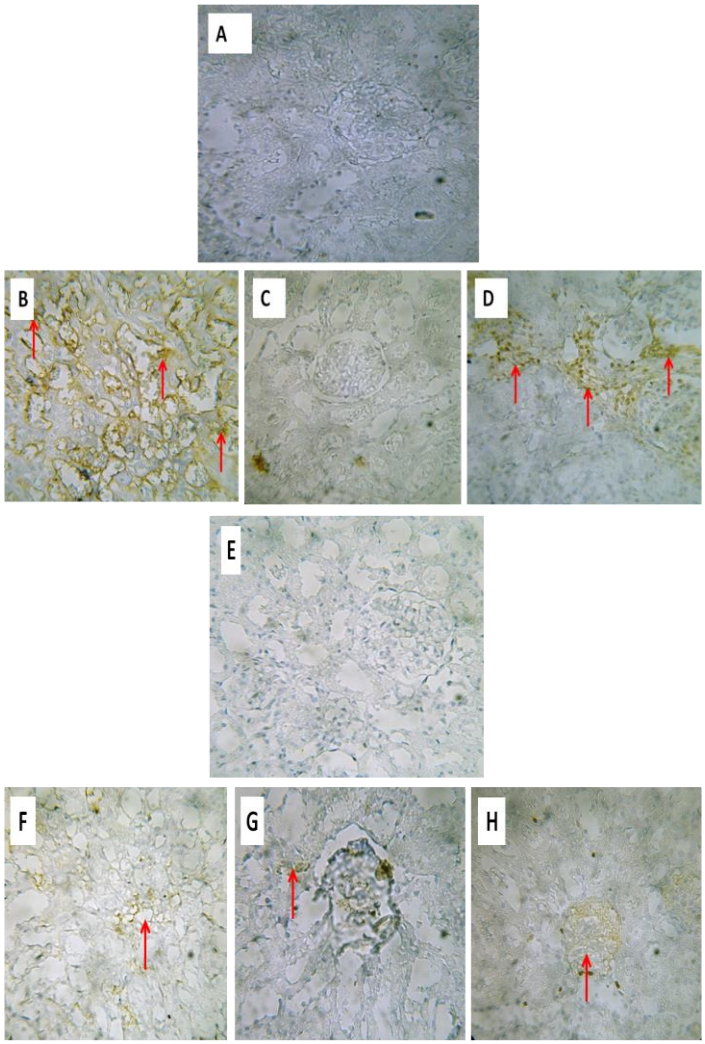

Figure (4): Immunohistochemical staining of inducible nitric oxide synthase (iNOS) (↑) in the rat kidney of different experimental groups (X 400). **A:** Control rat showing focal very poor staining with iNOS in convoluted tubular cells. **B:** Cisplatin treated rat showing diffuse iNOS staining in convoluted tubular cells (↑). **C:** γ-irradiated treated rat showing focal poor staining with iNOS in convoluted tubular cells. **D:** Cisplatin, γ-irradiated treated rat showing diffuse, intensive iNOS expression (↑) in convoluted tubular cells. **E:** GA treated rats showing negative diffuse staining with iNOS in convoluted tubules. **F**: GA, cisplatin treated rat showing poor diffuse staining with iNOS (↑) in convoluted tubules. **G**: GA, γ-irradiated treated rat showing poor diffuse staining with iNOS (↑) in convoluted tubules. **H:** GA, cisplatin and γ-irradiated treated rat showing poor diffuse staining with iNOS (↑) in convoluted tubules.

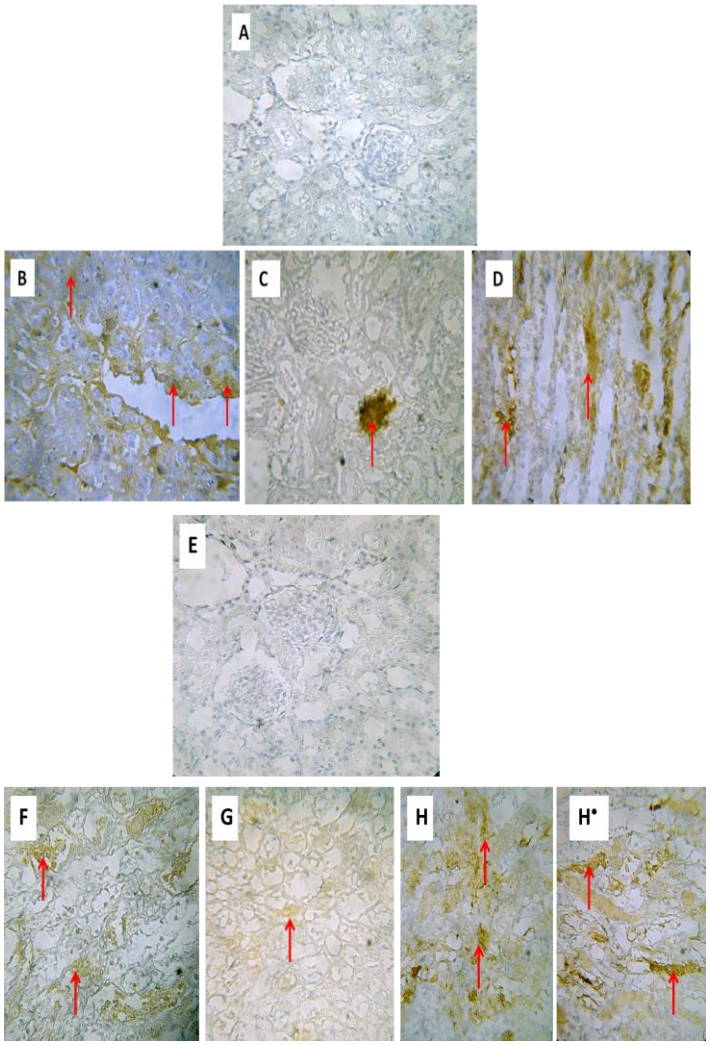

Figure (5): Immunohistochemical staining of endothelial nitric oxide synthase (eNOS) (↑) in the rat kidney of different experimental groups (X 400). **A:** Control rat showing focal very poor staining with eNOS in convoluted tubular cells. **B:** Cisplatin treated rat showing diffuse eNOS staining in convoluted tubular cells (↑). **C:** γ-irradiated treated rat showing focal poor staining with eNOS in convoluted tubular cells (↑). **D:** Cisplatin, γ-irradiated treated rat showing diffuse, intensive eNOS expression in convoluted tubular cells (↑). **E:** GA treated rats showing negative diffuse staining with eNOS in convoluted tubules. **F:** GA, cisplatin treated rat showing poor or less diffuse staining with eNOS (↑) in convoluted tubules. **G**: GA, γ-irradiated treated rat showing poor diffuse staining with iNOS (↑) in convoluted tubules. **H &H•:** GA, cisplatin and γ-irradiated treated rat showing poor diffuse staining with eNOS (↑) in convoluted tubules.

NO is a highly unstable, free radical gas synthesized from L-arginine mediated by NO synthase [46]. The increased NO production after iNOS induction can lead to direct DNA damage, mitochondrial membrane damage, or apoptosis. Although NO is a vasodilator molecule, excess production may lead to dose-dependent apoptotic or necrotic injury [47].

An increase in the iNOS expression level in tubular epithelial cells developing damage and increased iNOS expression parallel

to apoptotic injury as well as necrotic injury [48, 43] were determined. Acute nephrotoxicity was confirmed by pathological changes including swelling and vacuolation of the lining endothelium of the glomerulus tuft as well as tubular degeneration and dilation of the blood vessel with focal minute haemorrhage in the cortical portion. Endothelial NO may have a beneficial role as a vasodilator by inducing an increase in renal blood flow and in glomerular filtration in these animals. Excessive NO production can lead to cytotoxic injury. Peroxynitrite anion formation, protein tyrosine nitration, and hydroxyl radical production may be responsible for the evolution of the renal injury induced by cisplatin [43].

Essential antioxidants are either endogenous or exogenous. They are typically categorized as free radicals scavengers and protective antioxidants. GA acts as an antioxidant that modulates inflammatory and/or immunological processes. The cytoprotective effects of GA against cisplatin-induced nephrotoxicity and cyclophosphamide-induced urinary bladder cytotoxicity in rats have been ascribed to a scavenging action against ROS [26]. Daily treatments of rats with GA for two weeks significantly reduced serum creatinine, urea and glucose levels and renal contents of MDA, NO, TNF-α, iNOS and eNOS as well as MPO activity. Meanwhile, an improvement in renal GSH content in groups receiving GA was observed when compared to respective control values.

Effect of GA on renal iron (Fe), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), manganese (Mn), selenium (Se) and platinum (Pt) contents in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Combined treatment of cisplatin and γ-irradiation-induced a reduction in renal Fe, Cu, Mn, Ca and Se contents whereas Zn and Mg contents were unchanged. On the other hand, cisplatin alone or combined with γ-irradiation-induced more retention of platinum in renal tissues. Administration of GA significantly modulated these alterations in renal trace element contents ***(Table 2)*.**

Concentrations of essential trace elements in gum Arabic:

Results shown in **Table (3)** revealed the high contents of essential trace elements in GA. The concentrations trace elements were in the following order:

Mg > Ca > Zn > Fe > Mn > Cu > Se

Table (3): Concentration levels of trace elements in gum Arabic.

Element	**Concentration in GA (mg/L)**
Fe	31.45 ± 2.99
Cu	1.02 ± 0.03
Zn	46.63 ± 3.01
Ca	2483 ± 137.50
Mg	2611 ± 161.90
Mn	2.65 ± 0.13
Se	0.28 ± 0.02

Each value represents the mean of 6 samples ± S.E of the mean.

In the present study, combination of cisplatin and γ-irradiation induced alteration in renal trace elements, manifested by declined levels of Cu, Mn, Ca, Mg, Se and Fe contrary to Zn which was slightly increased and Pt which was retained in renal tissues. Many investigators observed trace element alterations after whole body irradiation [49, 50], and cisplatin administration [51]. The authors attributed the changes in Cu, Mn and Zn to the excess utilization of Zn, Mn and Cu enzymes after irradiation or due to *denovo* synthesis of metalloenzymes required for utilization of oxygen and prevention of superoxide anion radical accumulation. Rats supplemented with GA which represents an excellent source of metalloelements (Fe, Cu, Zn, Ca, Mg, Mn and Se), could attenuate the changes in renal contents induced by γ-irradiation and cisplatin treatment. These metalloelements are involved in multiple biological processes which correlate with the antioxidant capacities and the induction of endogenous metalloelement dependant enzymes. These enzymes play important roles in preventing the accumulation of pathological concentration of oxygen radicals or in repairing damage caused by irradiation injury [52].

V. CONCLUSION

Based on the results obtained in the current study, it appears that, GA attenuated the severity of biochemical disorders in renal tissues. This is mainly attributed to its free radical scavenging ability, high contents of bioactive components and essential trace elements in addition to antioxidant properties, implying minimization of lipid peroxidation and cytokines and enhancement of GSH contents.

Table (2): Effect of gum Arabic (GA) on renal iron (Fe), copper (Cu), zinc (Zn), calcium (Ca), magnesium (Mg), manganese (Mn), selenium (Se) and platinum (Pt) contents in rats subjected to cisplatin and/or γ-irradiation-induced nephrotoxicity.

Groups / *Parameters*	***Normal***	***GA*** (7.5%g/kg)	***Cisplatin*** (7.5mg/kg)	*GA* + ***Cisplatin***	***γ-radiation*** (6.5 Gy)	***GA*** + ***γ-radiation***	***Cisplatin*** + ***γ-radiation***	***GA*** + ***Cisplatin*** + ***γ -radiation***
Fe (µg/g)	62.80 ± 0.65	60.08 ± 1.46	* 45.10 ± 2.31	*b 50.45 ± 0.57	@ 69.66 ± 1.77	b 53.15 ± 2.26	*b 48.51 ± 0.76	b 53.97 ± 4.44
Cu (µg/g)	4.73 ± 0.29	4.60 ± 0.22	* 3.15 ± 0.19	* 3.11 ± 0.08	4.01 ± 0.45	4.40 ± 0.32	* 3.35 ± 0.20	* 3.28 ± 0.22
Zn (µg/g)	20.29 ± 0.53	20.90 ± 0.85	23.35 ± 0.90	* 22.87 ± 0.25	21.30 ± 0.50	23.72 ± 1.39	24.08 ± 0.50	24.42 ± 1.52
Ca (µg/g)	83.91 ± 3.15	82.09 ± 4.85	78.79 ± 5.75	b 65.62 ± 5.13	97.79 ± 8.50	*@b 48.26 ± 1.56	*@b 54.36 ± 3.94	c 82.73 ± 2.60
Mg (µg/g)	317.80 ± 15.43	277.20 ± 10.59	267.40 ± 16.75	263.60 ± 11.45	319.20 ± 24.40	*b 196.10 ± 11.35	252.70 ± 13.58	284.40 ± 28.80
Mn (µg/g)	1.93 ± 0.09	* 1.29 ± 0.06	* 1.24 ± 0.04	*b 0.98 ± 0.05	* 1.55 ± 0.04	* 1.47 ± 0.10	*b 1.14 ± 0.07	*b 1.07 ± 0.09
Se (µg/g)	0.56 ± 0.01	* 0.44 ± 0.02	* 0.36 ± 0.01	*b 0.43 ± 0.01	@ 0.55 ± 0.01	0.45 ± 0.02	*b 0.44 ± 0.03	*b 0.42 ± 0.02
Pt (µg/g)	0.02 ± 0.00	N.D	* 0.41 ± 0.03	*@ 0.26 ± 0.01	N.D	N.D	*@ 0.30 ± 0.02	*@ 0.24 ± 0.01

Each value represents mean ± S.E of the mean. Statistical analysis was carried out by one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.
*Significantly different from normal group at $p < 0.05$.
@Significantly different from cisplatin group at $p < 0.05$.
bSignificantly different from γ-irradiated group at $p < 0.05$.
cSignificantly different from cisplatin and γ-irradiated group at $p < 0.05$.
N.D: not detectable.

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