MJMR, Vol. 34, No. 1, 2023, pages (114-125).

Open Access
ISSN:2682-4558

Research Article

Reno-protective Role of Ginseng in Counteracting the Long-term Omeprazole Induced Adverse Effects in Albino Rats via Modulation of Inflammation, Apoptosis and Fibrosis

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DOI: 10.21608/mjmr.2023.182039.1255

Abstract

Background: Omeprazole has long-term use adverse effects even though are commonly used. The study aimed at evaluation of the possible renal protective effect of ginseng against the long-term omeprazole-induced adverse effects. **Methods:** Forty albino rats were divided into: C group (control), G group (ginseng), OM group (omeprazole) and OM-G group (omeprazole-ginseng). Renal functions and histological renal tissues were investigated. **Results:** The OM group had significantly high levels of urea, creatinine and 24-hours protein urea. A significant glomerular, tubular and interstitial injury was observed; congestion, inflammatory cell infiltration, tubular damage, increased collagen deposition and significant increases in COX-2 and caspase-3 immune expression. While adding ginseng with omeprazole resulted in marked improvement in these changes. **Conclusion:** Long-term use of omeprazole induced renal functional and morphological changes through induction of inflammatory reaction, fibrosis, cellular degeneration and apoptosis. By adding ginseng, these effects were ameliorated through its anti-fibrotic, anti-inflammatory, and anti-apoptotic effects.

Keywords: Ginseng, Inflammation, Fibrosis, COX-2, Caspase-3.

Introduction

Chronic kidney disease (CKD) is characterized by progressive biochemical and physiological deteriorations that affect body systems[(1)]. Unfortunately, the use of drugs that were considered safe has been detected as a possible cause of renal damage[(2)]. Among these drugs, proton pump inhibitors (PPI) which are highly prescribed worldwide shown to be associated with the occurrence of acute interstitial nephritis, reduction of glomerular filtration rate and the development of CKD[(3)]**.** Omeprazole is the prodrug of this family and is considered the first choice for most acid-related gastrointestinal disorders[(4)]. Recently, it has been increasingly suspected to induce acute renal failure and the development of CKD [(1)] among older patients[(5)] or even adolescents[(6)]. The association between PPI use and CKD development is a recent and not fully understood topic [(1)] [(7)]. Yet few studies have been done about the omeprazole effect on the kidney of experimental animals to study the renal structural changes.

Lately, complementary and alternative medicines; ginseng is the most commonly used, have been rapidly growing[(8)]. It has been widely used in the Western world for its tonic and various therapeutic effects[(9)]. It is a potent antioxidant herbal medicine effective for reducing lipid peroxidation and subsequent tissue damage. It also displayed anti-inflammatory properties, anti-apoptotic

and immunostimulatory effects[10, 11]. It is reported to be effective in the treatment of various disorders including treatment of chemotherapy-induced side effects[12, 13].

Therefore, this study aimed to experimentally evaluate the possible protective role of ginseng against the potential structural changes in the renal cortex of albino rats following long-term omeprazole administration.

Material and method

Animals and experimental design:

This experiment was done and approved by the ethical committee for animal handling for research according to international guidelines (Act 1986) in the Faculty of Medicine, Minia University.

Thirty-two adult male rats (6-8 weeks and 150-200 grams) were housed in clean plastic cages at 25 °C and normal light/dark cycles with ad libitum access to water and food for 2 weeks before the study. Rats were divided into 4 groups (8 rats each) and received the following for 12 weeks:

- The C group (control group): standard rat diet and clean water.
- The G group (ginseng group): 100 mg/kg/day of dried roots of Panax ginseng (GINSANA®, EPICO Pharmaceuticals, Cairo, Egypt) freshly dissolved in 100ml distilled water by oral gavage in a single daily dose [14].
- The OM group (omeprazole group): 20 mg/kg/day omeprazole (Pepzol MR®, Hekma Pharmaceuticals, Cairo, Egypt) freshly dissolved in distilled water by oral gavage in a single daily dose[15].
- The OM-G group (omeprazole-ginseng group): ginseng and omeprazole; as mentioned in the G group and OM group, simultaneously.

Biochemical study:

- Assessment of the 24-hours protein in urine: rats were separated singly in metabolic cages and the 24-hours urine was collected just before the end of the experiment and urine proteins were measured[16].
- Evaluation of serum urea and creatinine levels: blood samples were drawn by the end of the experiment, allowed to stand to clot (30 minutes), and centrifuged (15 minutes) to obtain sera. Commercial creatinine[17] and urea kits were used[18].

Histological procedures and image capture:

- Rats were decapitated under light halothane anesthesia and kidneys were obtained. Tissues were fixed, paraffin-embedded and sectioned into 5-μm sections that used for:
- Hematoxylin & eosin (H&E), Periodic acid Schiff's (PAS) and Masson trichrome staining**.**
- Immunohistochemical study**:** The endogenous peroxidases were quenched by H_2O_2 and washed in tris buffer saline, incubated with the diluted 1^{ry} antibodies; cleaved caspase-3 (1:200) and the ready used COX-2 antibody. After that, sections were incubated in biotinylated goat anti-rabbit 2ry antibody (1:1000), Vecta-stain ABC kits (Avidin, Biotinylated horseradish peroxidase Complex) and DAB was added for 5-10 min.
- A light microscope connected to a digital camera (Olympus, Japan) was used for examination and capturing images.

Morphometrical evaluation:

Ten nonoverlapping fields from 3 sections for each rat were examined at a power of X 400.

- **Histopathological scoring (21) and evaluation of alterations in the basal membranes and tubular brush borders in PAS stained sections(22):** Score 0: normal unaltered structure. Score 1: in between normal and mild level. Score 2: (mild level) less than 25 %. Score 3: (moderate level) less than 50 %. Finally, score 4: (severe level) less than 75 % of the total fields examined.
- **Scoring of fibrosis using Masson trichrome stained sections(23):** Score 0: <10% , Score 1: 10–25%, Score 2: 26%–50%, and Score 3: >50% of renal cortex involved by interstitial fibrosis**.**

- **Measuring area fraction of COX-2 and activated caspase-3 immune-reactivity (24):** image J 22 software (open-source Java image processing program) was used in a standard measuring frame/5 images for each group regardless of the intensity of staining**.**

Statistical analysis:
The SPSS (IBM corp., Version 20) program was used for the statistical analysis. Numerical data were represented as means ± standard error of the mean (SEM). ANOVA test and the Tukey–Kramar post-analysis test were performed. When a p-value was ≤ 0.05, it was considered significant.

Results

Biochemical results:
The serum urea, creatinine and the total 24-hours urinary proteins had insignificant differences between the control and G groups (all $p>0.05$). The OM group showed significant increases compared to the C and the G groups (all $p<0.001$). However, the OM-G group had a significant decrease in its levels compared to OM- group (all $p<0.001$) (Table 1).

H&E results and histopathological scoring:
Both control and G groups showed normal renal cortical structure. The cortex was formed of renal corpuscles, proximal and distal convoluted tubules. Each corpuscle consisted of glomerular capillaries surrounded by a Bowman's capsule with a space between its 2 layers. The distal convoluted tubules had larger regular distinct lumina than the proximal convoluted tubules and had the macula densa cells close to the renal corpuscles. The group received omeprazole had variable and patchy histological changes including glomerular, tubular and interstitial injury with marked vascular congestion. Numerous corpuscles were congested or distorted with glomerular vacuolation and widening of the Bowman's space. Renal tubules showed marked distortion; the epithelial lining was either vacuolated or showed apoptotic figures (deeply acidophilic cytoplasm and dark pyknotic nuclei), had reduced cell height, or even flattened and desquamated cells with cellular debris appeared in the lumina. Inflammatory cellular infiltration was also observed. But sections from the OM-G group showed that coadministration of ginseng markedly ameliorated the damaging effects of long-term use of omeprazole. Renal corpuscles appeared more or less normal with mild congestion of glomerular capillaries and of the proximal and distal convoluted tubules retained their apparently normal epithelium (Fig.1).

Results of the PAS staining:
A normal positive PAS reaction was observed in the control and G groups either in the basement membranes of glomerular blood capillaries, parietal layers of Bowman's capsules, proximal and distal convoluted tubular cells. The apical brush borders of proximal and distal convoluted tubular cells appeared intact. The OM group had significantly reduced PAS reaction due to partial or complete loss of the brush borders, interrupted or thin basement membranes. However, in the OM-G group, cells were mostly preserved PAS reactions except for only a few areas of brush borders of some renal tubules (Fig. 2).

Results of Masson trichrome staining:
Sections from both the control and G groups showed normal fine strands of collagen fibers. The amount of collagen in OM group were significantly increased compared to the control groups and significantly decreased compared to the OM group (Fig.2).

Results of immunohistochemical staining

- Regarding the COX-2 immune-expression, both control and G groups showed insignificant difference ($p=0.998$) which appeared as a faint cytoplasmic expression in few macula densa cells. A significant increase was found in the OM group compared to the other groups (all $p<0.001$) where the expressions were seen in macula densa, proximal and distal convoluted tubules and some glomerular cells. However, there was a significant decrease in the OM-G group if compared to the OM

group (p<0.001) which restricted to macula densa cells (Fig.3).

- Regarding the activated caspase-3 immune-expression, the control and G groups showed no detectable expression while the OM group showed a significant increase in immune expression compared to the other groups (all p<0.001). Meanwhile, the OM-G group had a significant decrease compared to the OM- group (p<0.001) (Fig.3).

Table 1: Serum urea (mg/dl), serum creatinine (mg/dl) and 24-hours urinary protein (gm/dl) levels in the studied groups (n=8)

		C group	G group	OM group	OM-G Group
Serum urea (mg/dl)					
Range *Mean ± SD*		(23.8-28) 25.9±1.6	(25.2-28.6) 27.4±1.2	(44.1-55.2) 49.1±3.6	(35.1-39.2) 37.3±1.3
P value	**ANOVA**	***< 0.001****			
	Post hoc				
	C group		*0.549*	***< 0.001****	***< 0.001****
	G group			***< 0.001****	***< 0.001****
	OM group				***< 0.001****
Serum creatinine (mg/dl)					
Range *Mean ± SD*		(0.4-0.8) 0.6±0.1	(0.5-1.2) 0.7±0.2	(8.5-11.7) 9.8±1.1	(4.5-6.2) 5.1±0.6
P value	**ANOVA**	***< 0.001****			
	Post hoc				
	C group		*0.795*	***< 0.001****	***< 0.001****
	G group			***< 0.001****	***< 0.001****
	OM group				***< 0.001****
24-hours urinary protein (gm/dl)					
Range *Mean ± SD*		(0.3-0.56) 0.43±0.1	(0.25-0.7) 0.49±0.15	(2.1-2.8) 2.46±0.24	(0.7-1.4) 1.03±0.22
P value	**ANOVA**	***< 0.001****			
	Post hoc				
	C group		*0.941*	***< 0.001****	***< 0.001****
	G group			***< 0.001****	***< 0.001****
	OM group				***< 0.001****

*: Significant difference at p-value < 0.05

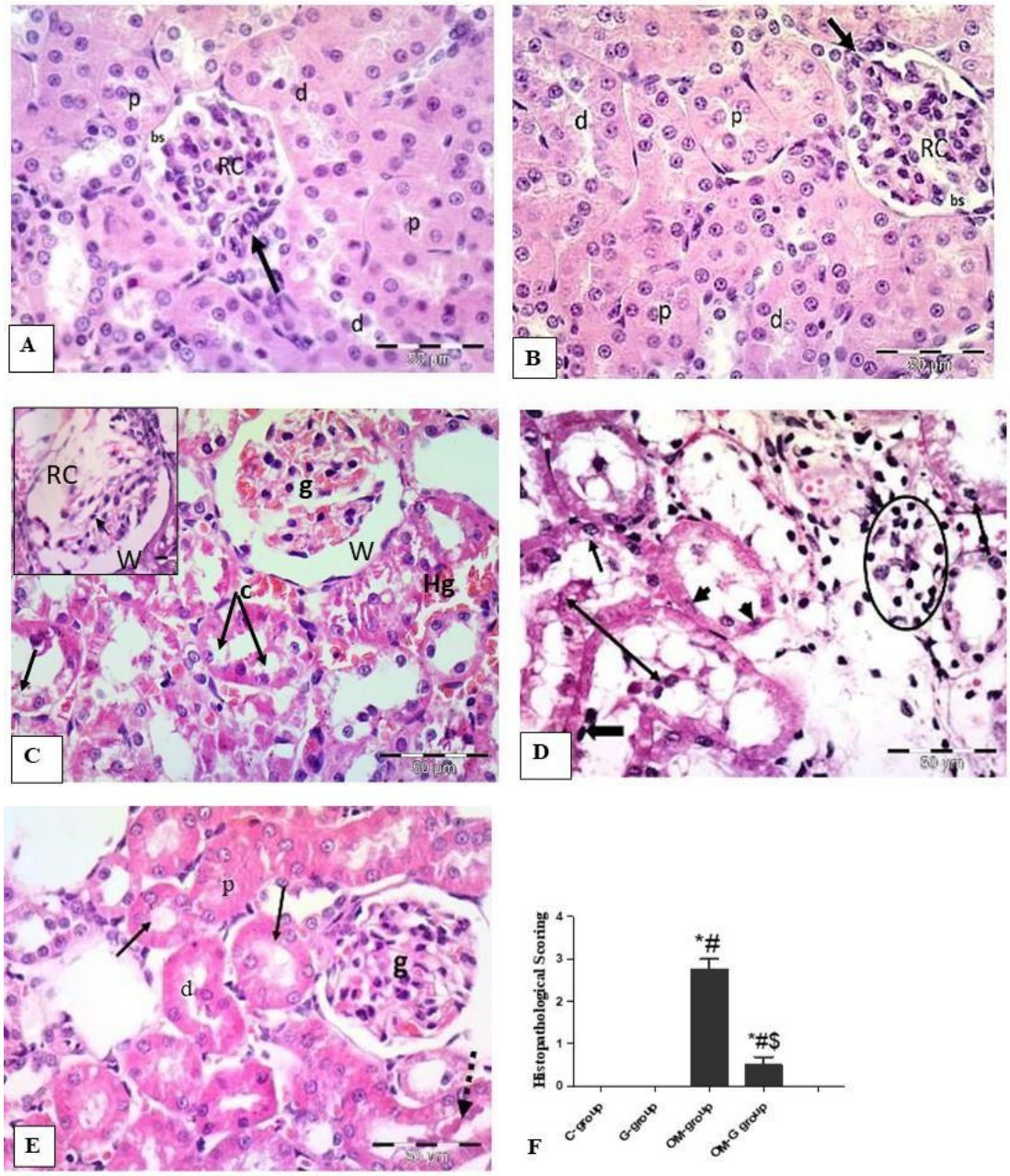

Fig. 1: Photomicrographs of renal cortices from:
- The C group (A) and G group (B) showing normal renal cortical structure. Renal corpuscles; RC, bowman's space; bs, proximal convoluted tubules; p, distal convoluted tubules; d, and macula densa; arrow.
- The OM group (C & D) showing glomerular (g) and interstitial vascular congestion (c), interstitial hemorrhage (Hg), and vacuolation of tubular cells (arrow). Inset: deformed renal corpuscle (RC), and widening of Bowman's space (W). Notice tubular cell distortion; flattening (thin arrows) or desquamation with cellular debris in lumina (double head arrow), apoptotic figures (thick arrow) and inflammatory cell infiltrate (circle).
- The OM-G group (E) showing restoration of normal cortex appearance with normal tubular epithelium (arrows), minimal hyaline casts (dashed arrow) and mild congestion of glomerular capillaries (g).

H&E X 400

- Histopathological scoring of cortical morphological changes (F) in the studied groups (n=8), *: significant vs control, #: significant vs G group, $: significant vs OM group at $p<0.05$.

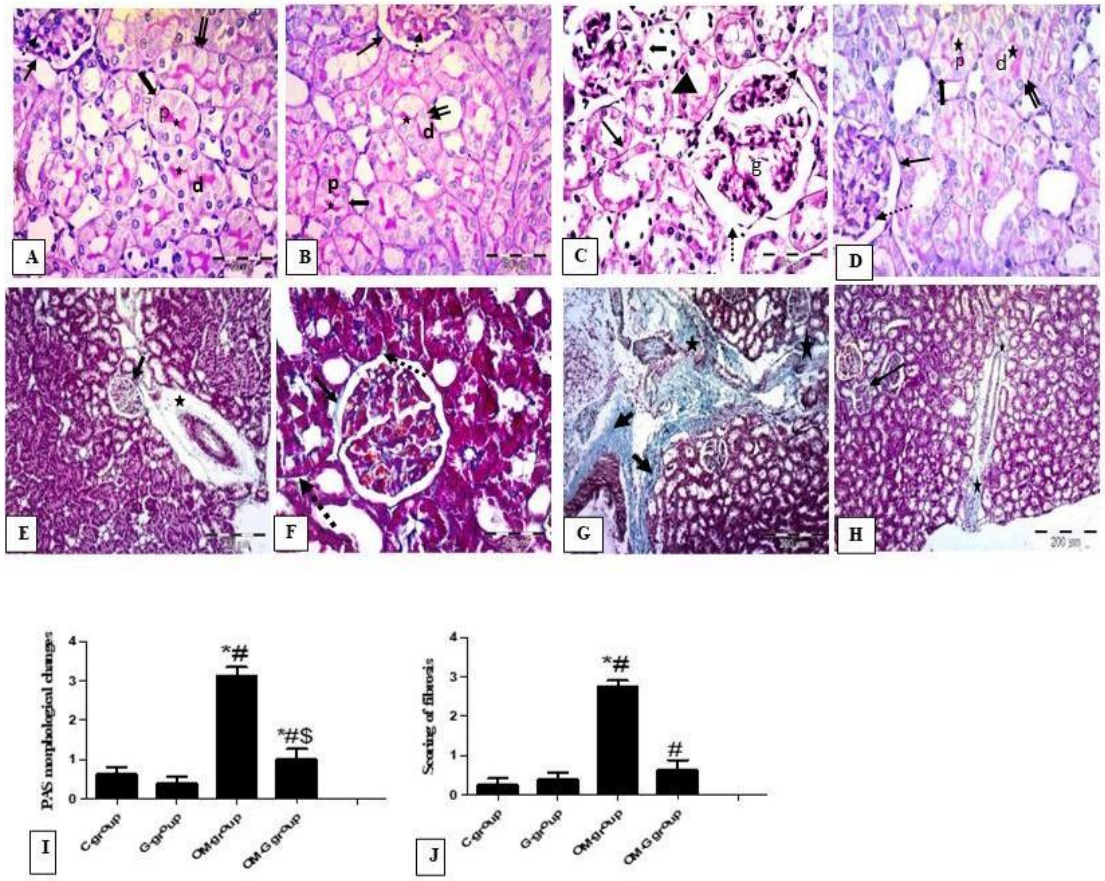

Fig. 2: Photomicrographs of renal cortices from:
- The C group (A) and G group (B) showing positive PAS reaction in the BMs of glomerular capillaries (dotted arrow), parietal layers of Bowman's capsules (thin arrow), PCTs (thick arrow), and DCTs (double arrow) cells. Notice brush borders (stars) of tubules. The OM group (C) showing partial (thin arrows) or complete loss (thick arrows) of the brush borders. Notice interruption of basement membrane (dashed arrow). The OM-G group (D) showing preserved most brush borders (stars), continuous BMs of glomerular capillaries (thin arrow), parietal layers of Bowman's capsule (dotted arrow), proximal (thick arrows) and distal (double arrows) convoluted tubular cells.

PAS X400

- The C group (E) and G group (F) showing fine strands of collagen fibers surrounding the glomeruli (arrow), between tubules (dashed arrows), and around the renal calyx (star). The OM group (G) showing increased amount of collagen around the renal calyx (thick arrows) and in between lobules (stars). The OM-G group (H) showing reduction of collagen surrounding the renal calyx (stars) and the glomeruli (arrow).

Masson trichrome X100; FX400

- Scoring of cortical morphological changes in the PAS-stained sections (I) and fibrosis in the Masson trichrome sections (J) in the studied groups (n=8), *: significant vs control, #: significant vs G group, $: significant vs OM group at $p<0.05$.

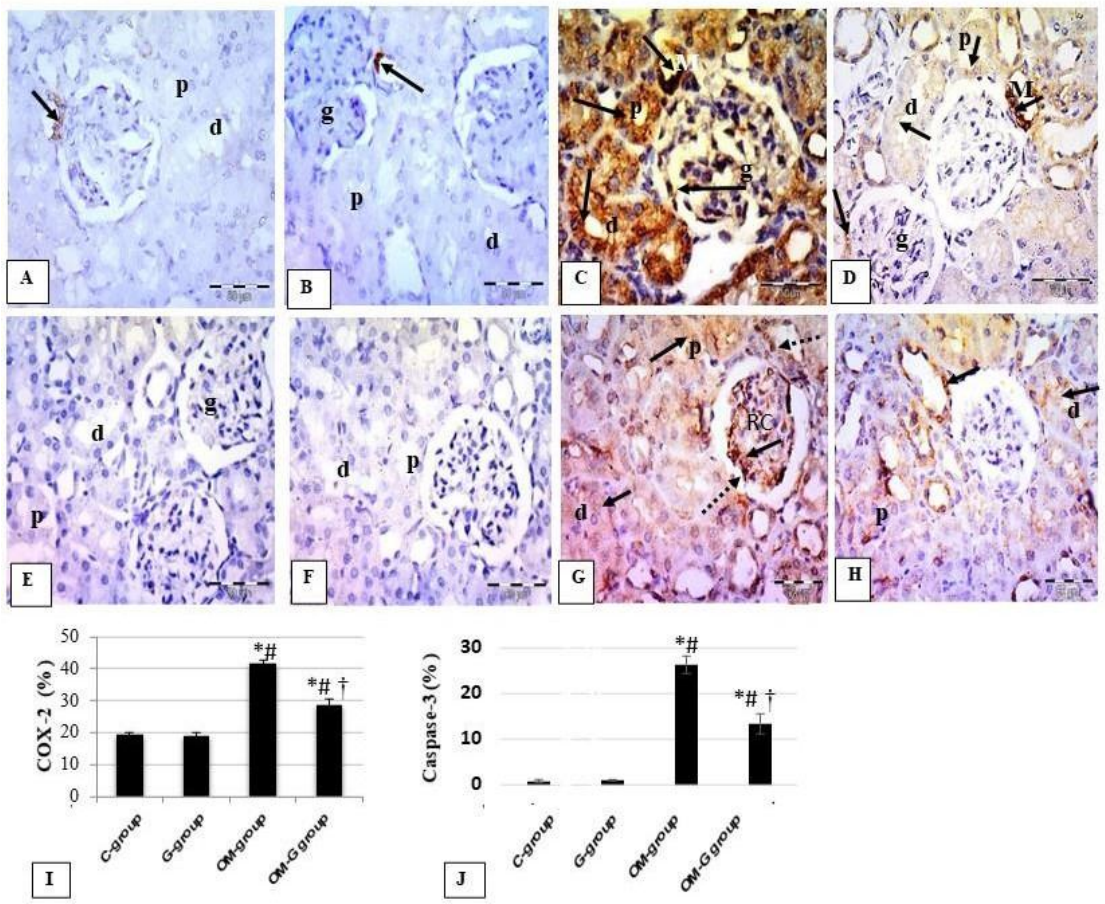

Fig. 3: Photomicrographs of immunostained renal cortices for COX-2 (A-D) and caspase-3 (E-H) from:

- The C group (A) and G group (B) showing COX-2 few immune-expression in macula densa cells (arrow). The OM group (C) showing increased expression (arrows) in the proximal (p) and distal (d) convoluted tubule cells, and some glomerular cells (g). The OM-G group (D) showing decreased expression (arrows) in most tubules with deep expression (dotted arrow) in macula densa cells (M).
- The C group (E) and G group (F) showing negative caspase-3 immune reactivity. The OM group (G) showing positive cytoplasmic immune expression (arrows) in renal corpuscle (RC), proximal (p) and distal (d) convoluted tubules. Notice some nuclear expression (dotted arrows). The OM-G group (H) showing decreased expression in some tubular cells (arrows).
- The mean area fraction of COX-2 (I) and caspase-3 (J) immune-reactivity in the studied groups (n=8), *: significant vs control, #: significant vs G group and †: significant vs OM group at $p<0.05$.

Immunohistochemistry counterstained with H x400

Discussion

With the widespread use of PPIs, several studies are concerned about the safety of PPIs treatment. Among these, renal injury following PPI therapy was a hot issue[7]. In our study, after long-term omeprazole administration, serum urea and creatinine levels showed marked increases**.** The interstitial inflammation could stimulate the accumulation of extracellular matrix (ECM) which in turn lead to impairment of renal functions[25]**.** The inflammatory mediators cause endothelial cell activation, vascular permeability, and proteinuria[26]**.** Thus, the inflammation and structural changes observed in the OM group might be attributed to the impaired renal functions which were in agreement with findings of another research[27]**.** Korean red ginseng is one of the best-selling dietary supplements and its individual constituents enhance renal function[28]. Thus, it was of interest to investigate the protective effect of ginseng co-administration with omeprazole which already resulted in a significant improvement in renal functions in our results.

In line with Mubeen et al.,,[29]**,** our results showed that the long-term use of omeprazole induced variable and patchy morphological changes in the renal histological structure and demonstrated significant glomerular and tubulointerstitial injury. Omeprazole had toxic effects on the vasculature of the kidney which was demonstrated by glomerular congestion and atrophy in addition to the stromal hemorrhage and congestion resulting from weakness of renal vasculature structure**.** Others[6, 23] added that the accumulation of inflammatory cells and the generation of cytokines from damaged tubules resulted in glomerulo-tubular disconnection and collapse of glomeruli**.** In contrast, ginseng improves lipid metabolism and produces beneficial effects on vascular endothelial function[11].

The cytoplasmic vacuolation, degeneration, necrosis of tubular cells could be attributed to disturbed ion transport system or occurs as a cellular defense mechanism against injurious materials which segregated in vacuoles interfering with cellular metabolism[30]**.** Cortical tubular cells predominantly contain cytochrome P-450 which is responsible for omeprazole metabolism [31]**.** Hence, omeprazole has a high affinity to the tubular cells leading to the release of free oxygen radicals that covalently bind to cellular macromolecules especially polyunsaturated fatty acids, initiating autocatalytic lipid peroxidation, DNA damage, protein degeneration and apoptosis[32]**.** Taken together, these might interpret the tubular degenerations and the increased apoptotic figures observed in the tubular cells in the OM group. Ginseng, as a free-radical scavenger and lipid peroxidation inhibitor[33]**,** resulted in amelioration of the damaging effects of prolonged omeprazole in this study due to its antioxidant property. This ameliorative effect might be also due to what was mentioned by Shen et al.,, [34] who owed that to the ability of ginseng to encourage cell proliferation and improve the survival rate of newly formed cells through activation of glucocorticoid response**.**

An interesting reciprocal relationship between proteinuria and tubular injury was evidenced where the local inflammatory cell infiltration initiates the release of inflammatory cytokines and growth factors such as the transforming growth factor-β and vascular endothelial growth factor-A[35]. This might be a major cause of nephrin (an important protein of the slit diaphragm of podocytes that has anti-apoptotic signaling properties) downregulation and occurrence of proteinuria[36]**.** On the other hand, the filtered macromolecules provoke harmful effects on tubular cells as energy depletion and lysosomal rupture leading to a cascade of events that end in cell injury[37]**.** Chronic renal damage is mostly assessed by visual estimation of the extent of interstitial fibrosis and the fraction of tubular damage[38]. The observed interrupted tubular and glomerular basement membranes and also the variable damages of the tubular brush borders were in line with the study of Berney- Meyer and his colleagues [27]**.** The renal tubular brush border is evidenced in the peroxidation of lipids. Thus changing the properties of the biological membranes

leads to severe cell injury leading to loss of its apical brushing[39]. Using ginseng markedly improved these changes due to that ginseng protect cells and tissues from the destructive effects of the free radicals[14] and also the lipid peroxidation inhibitory effect of ginseng[33].

Collagen deposition clearly occurred in the OM group could be explained as inflammatory cells might be the physio-pathological link between tubular epithelial cell injury and renal fibrosis. The inflammatory cells are important sources of cytokines and growth factors. These mediators play a critical role in the progress of interstitial fibrosis[36]. In addition, several experimental studies and clinical observations proved that the process of fibrosis occurs as a result of altered crosstalk between the tubular cells and the interstitial fibroblasts[40]. Under certain pathological conditions; according to the Epithelial-Mesenchymal Transition phenomenon, a considerable number of the interstitial fibroblasts could be originated from the neighboring tubular cells leading to more production of the ECM[41]. Entertainingly, in our study, coadministration of ginseng with omeprazole resulted in a reduction of collagen fiber deposition might be through the correction of the altered crosstalk.

The OM group showed a significant increase in COX-2 expression which might be due to the observed lymphocytic infiltration and inflammatory induction by omeprazole. An idiosyncratic immune reaction secondary to a cell-mediated process induced by omeprazole leads to renal injury[42]. The decreased inflammatory cell infiltration and the significant decrease of COX-2 expression in the OM-G group in our results indicated an anti-inflammatory effect of ginseng. This was decided previously as ginseng radix and its compounds are implicated in reducing inflammation[43].

In the current study, the apoptotic process was clearly observed in the OM group while ginseng co-treatment decreased it significantly. Omeprazole decrease levels of the anti-apoptotic proteins as Bcl-2 and Bcl-XL which control cell survival[44]. While ginseng has a potent anti-apoptotic effect by increasing expression of Bcl-2 (the anti-apoptotic gene) which might antagonize the previously mentioned mechanism of omeprazole-induced apoptosis. Jung et al.,,[45] proved that ginseng has a potent protective role on renal dysfunction through its antiapoptotic activities which explain the significant decrease of apoptosis by ginseng co-administration in this study.

These findings draw attention to the fact that long-term omeprazole nephrotoxicity is a risk for a progressive loss of renal function and normal structure and in turn, it might be a cause of CKD. Thus, it might be important to add ginseng when the prescription of omeprazole or PPI is required for a long time to avoid occurrence of CKD.

Conclusion:

The long-term use of omeprazole induced functional and morphological changes in the kidney. All through induction of inflamamatory reaction, fibrosis, cellular degeneration and apoptosis. By adding ginseng, these effects were ameliorated through its anti-fibrotic, anti-inflammatory, and anti-apoptotic effects.

Acknowledgment:

The authors acknowledge the support of our preprint by **bioRxiv** [46].

Conflicts of interest:

The authors declare that there is no conflict of interest.

Funding:

The authors did not receive any grant.

Author contribution statement:

Abdel Dayem DA: performed the experimental lab work. El-Tahawy NF: writing of the manuscript. Ali AH: helped in experimental design and data analysis. Mahmoud AS: helped in the experimental design and writing of the manuscript.

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