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ORIGINAL PAPER

Protective effect of *Panax ginseng* against serum biochemical changes and apoptosis in kidney of rats treated with gentamicin sulphate

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Abstract The protective effects of *Panax ginseng* (PG) on gentamicin sulphate (GS) induced acute nephrotoxicity were investigated in rats. A total of 32 adult Sprague–Dawley rats were randomly divided into 4 equal groups and treated by intraperitoneous route for 10 days with: 0.5 mL of isotonic saline (group C), GS 100 mg/kg/day (group GS), co treatment PG (100 and 200 mg/kg/day) plus GS (100 mg/kg/day). After the last injection, kidney markers (urea, creatinine and blood urea nitrogen-BUN) and hepatic markers (aspartate aminotransferase-AST, alanine aminotransferase-ALT, gama glutamil transferase-GGT), and biochemical parameters were analyzed using diagnostic kits. Also, kidney changes were evaluated by immunohistochemical and stereological methods. GS treatment induced significant elevation ($P < 0.05$) in kidney and hepatic markers, most of biochemical parameters, and Bax immunoreactivity as well. However, co treatments with both doses of PG (100 and 200 mg/kg/day) significantly alleviated ($P < 0.05$) the GS-induced elevations and have partially protected rats from nephrotoxicity (reduction of kidney damage, and of urea, creatinine and BUN concentrations, and of apoptotic index). Both biochemical results and immunohistochemical evidence showed that administration of PG reduced the gentamicin-induced nephrotoxicity.

Keywords *Panax ginseng* · Gentamicin sulphate · Nephrotoxicity · Apoptosis

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Introduction

The results of scientific researches reported that radiation and environmental pollutants such as chemicals and drugs including antibiotics dramatically change the structures and functions of various cells or tissues, and induce some adverse effects such as insufficiency, tumor and cancer in the liver, kidney, heart and intestine tissues. The aim of treatment is to obtain preferential protection of healthy cells and tissues against injury inflicted by antibiotics, ionizing radiation or chemotherapeutic agents used to treat tumors, cancers and infections. To date, there are many

scientific researches for this purpose, but little success has been achieved (Forge and Schacht 2000).

Antibiotics are the cornerstone for the prevention and treatment of numerous infections, but they have some side effects. The more common side effects include that nausea, vomiting, diarrhea, abdominal pain, allergic reactions, fever, headache, confusion, dizziness and auditory impairment. The most widely used antibiotic for treatment of gram positive and gram negative infections in aminoglycoside category is gentamicin sulphate (GS). However, nephrotoxicity is major side effect of GS, accounting for 10–20% of cases of acute renal failure (Mattew 1992; Forge and Schacht 2000). The main mechanism of GS-induces nephrotoxicity is unknown; however, there may be several mechanisms have been shown both in vitro and in vivo studies to enhance the generation of reactive oxygen species (Balakumara et al. 2010). These include firstly, GS altered the function and structural damages of extracellular and intracellular membranes through binding to anionic phospholipids (Laurent et al. 1990), and secondly, GS cause ATP depletion from mitochondrial damage and/or inhibition of mitochondrial oxidative phosphorylation (Nakajima et al. 1994). On the other hand some researchers informed that GS act as an iron chelator, and that the iron-GS compound is a potent catalyst for Fenton's reaction (Priuska et al. 1998).

Apoptosis is a programmed form of cell death mediating the precisely controlled deletion of unwanted cells. External and internal factors stimulate and/or contribute apoptotic mechanisms that are believed to play a key role including caspase, Bax and Bcl-2 family. Especially Bax and Caspase enzymes play an important role in apoptotic process of the kidney tissues. Whereas, Bcl-2 is acts either antiapoptotic (Bcl-xL) or pro-apoptotic (Bcl-xS). Previous researchers have been reported that the stimulation of apoptosis is a significant cytotoxic mechanism of gentamicin in renal proximal tubular cells and mesangial cells (Martínez-Salgado et al. 2004; Servais et al. 2006). Finally Servais et al. (2006) and Denamur et al. (2011) reported that gentamicin induces apoptosis in LLC-PK1 cells of kidney with triggering of the mitochondrial pathway and activation of Bax. A major Bax activation pathway is the cytochrome *c*-initiated pathway in kidney cells, which is regulated by Bcl-2 family proteins, including Bcl-2, Bad and Bcl-xL (Saikumar and Venkatachalam 2003).

Although various natural and synthetic antioxidant agents in order to prevent or ameliorate kidney injury related to GS nephrotoxicity have been well used, there is no specific treatment against GS-induced nephrotoxicity. These antioxidant agents include *Spirulina platensis* (Karadeniz et al. 2008), lycopene (Karahan et al. 2005), resveratrol (Silan et al. 2007), green tea extract (Khan et al. 2009), taurin (Erdem et al. 2000), caffeic acid phenethyl ester (Vardi et al. 2005). *Panax ginseng* (PG) is a most popular phytotherapeutic agent in last year's, and it contains ginsenosides which are phenolic acids, flavonoids and triterpenoid saponins. These properties of the ginseng are thought to provide many beneficial effects against organ damages (Kitts and Hu 2000). Already in previous studies, show that PG has a various antiapoptotic effects that include in liver (Karakus et al. 2011), vascular smooth muscle (Xu et al. 2011), lung cancer (Wang et al. 2009), heart (Chen et al. 2011), hippocampus neuron (Zhu et al. 2003), and bone marrow stromal cells (Qiang et al. 2010). However there is no scientific information related to anti-apoptotic effects of PG in kidney induced by GS. Therefore, we aimed to investigate the protective effect of PG on kidney and liver markers, serum biochemistry and apoptosis in kidney tissue induced by GS in rats.

Materials and methods

Chemicals

Gentamicin sulphate and PG were purchased from Vetaş (İstanbul, Turkey) and trade cooperation from SGM (Ankara, Turkey), respectively. All other chemicals of analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO) or Merck (Darmstadt, Germany).

Animals, housing and experimental design

In this study, 32 healthy adult male Sprague–Dawley rats weighting between 200 and 250 g were used. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (22 ± 3°C) during the experimental period. All experimental procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Water and food were provided ad libitum. The animals were randomly divided into four equal groups (n = 8). In the control group (group C), rats received a daily intraperitoneal (i.p.) injection of 0.5 ml isotonic saline for 10 days. In the gentamicin sulphate (group GS) group, rats were received i.p. daily treated by GS doses 100 mg/kg/day for 10 days, whereas in the PG + GS groups, animals were simultaneously injected by the two drugs (PG: 100 and 200 mg/kg/day doses, respectively plus GS: 100 mg/kg/day) for 10 days.

Blood sampling and biochemical analysis

All rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly, and then killed by cervical dislocation 24 h after the last injection and trunk

blood was collected into tubes and centrifuged at 3,200*g* at 25 °C room temperature for 10 min. The sera were stored in the −20 °C freezer before they were analyzed. The kidney markers [creatinine, urea, blood urea nitrogen (BUN)], hepatic markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gama-glutamil transferase (GGT)], some biochemical parameters [total triglyceride, cholesterol glucose, total protein, calcium and phosphorus] were analyzed using diagnostic kits (IBL Chemical Co., Ankara, Turkey).

Immunohistochemical examination

The kidney tissues were fixed in 10 % buffered neutral formalin and embedded in paraffin. The paraffin blocks were cut 5–7 μm thick. Apoptotic cells were determined by immunohistochemical method (streptavidin–biotin-peroxidase staining). For immunohistochemical examination, primary antibodies; Bax (dilution: 1/50, Abcam, Cambridge), and Bcl-2 (dilution: 1/50 Santa Cruz) and biotinylated secondary antibody (Dako-Universal LSAB Kit-K0690) were used. The binding sites of antibodies were visualized with DAB (Sigma). All immunohistochemical evaluations were estimated via stereologic fractionator principle.

Stereological procedure

Stereology is a practical and reliable technique for extracting quantitative information about a three-dimensional material from measurements conducted with two-dimensional planar sections of the tissues. In all group sections, the numerical density of cells (according to the antibodies' positivity) was evaluated via optic fractionator frame method. Stereological analyses were performed in a stereology workstation consisting of a modified light microscope (Leica DM4000 B), a motorized specimen stage for automatic sampling (BioPrecision MAC 5000 controller system), a CCD color video camera (Optronics MicroFire), and stereology software (Stereo Investigator version 9.0, Microbrightfield, Colchester, VT). The unbiased counting frame-fractionator combination is a stereological method for counting cells in tissue sections (Howard and Reed 1998; Sterio 1984). In the study, we used the unbiased counting frame and fractionator methods to estimate numerical density of cells according to staining with Bax and Bcl-2 antibodies in the kidney both cortex and medulla. Each section was sampled using the fractionator principle of the stereology software. Cells were counted using a 40× Leica Plan Apo objective (NA = 1.40), which allowed accurate recognition. Each cell was counted by the stereology software according to the unbiased counting frame (Fig. 1). The numerical density was estimated according to the formula given below:

$$\mathrm{Nd} = \mathrm{TM}/(\mathrm{CFA} \times \mathrm{NSS}),$$

where Nd is numerical density, TM is total markers, CFA is counting frame area (XY) (μm^2), and NSS is number of sampling sites.

Statistical analysis

The histological and biochemical results were expressed as means in each group. For statistical analysis, differences between the groups were tested by analysis of variance followed by the Duncan post hoc test using SPSS software, version 17.0, for Windows XP (SPSS Inc.). Statistical significance was accepted for all tests at $P < 0.05$.

Results

Macroscopic results

No animal died in the experiment, and no major change in food and water intake was observed for rats of all groups. On autopsy, kidneys were slightly swollen and pale in GS treated group and this appearance was similar to observations made by Rouiller (1969) and Kotnis et al. (2004).

Effects of GS and PG treatments on kidney markers and kidney weights

The level of kidney markers and kidney weights of rats in control and all treated groups are showed in Table 1. GS treatment induced a significant increase in the relative weight of kidneys with respect to normal controls (0.48 ± 0.03 vs. 0.60 ± 0.04) ($P < 0.05$), whereas co treatment of PG (100 and 200 mg/kg/day) to GS-intoxicated rats was unable to diminish such increase (0.60 ± 0.04 vs. 0.55 ± 0.02 and 0.50 ± 0.03, respectively) ($P < 0.05$). As shown in table serum levels of creatinine, urea and BUN were significantly higher in the GS-treated animals when compared with the control group (0.40 ± 0.12 vs. 1.55 ± 0.25; 43.55 ± 4.75 vs. 96.25 ± 9.50, 13.80 ± 4.48 vs. 80.25 ± 5.40 respectively) ($P < 0.05$). Co-treatment of the animals with PG (100 and 200 mg/kg/day) significantly reduced the high level of serum creatinine, urea and BUN (1.55 ± 0.25 vs. 0.95 ± 0.15 and 0.45 ± 0.20; 96.25 ± 9.50 vs. 72.38 ± 5.75 and 40.80 ± 7.50, 80.25 ± 5.40 vs. 15.50 ± 5.25and 14.35 ± 6.50, respectively) ($P < 0.05$).

Effects of GS and PG treatments on hepatic markers

The activities of AST, ALT and GGT were estimated in serum samples as the liver function markers. Those results are shown in Table 2 for all groups. According to the

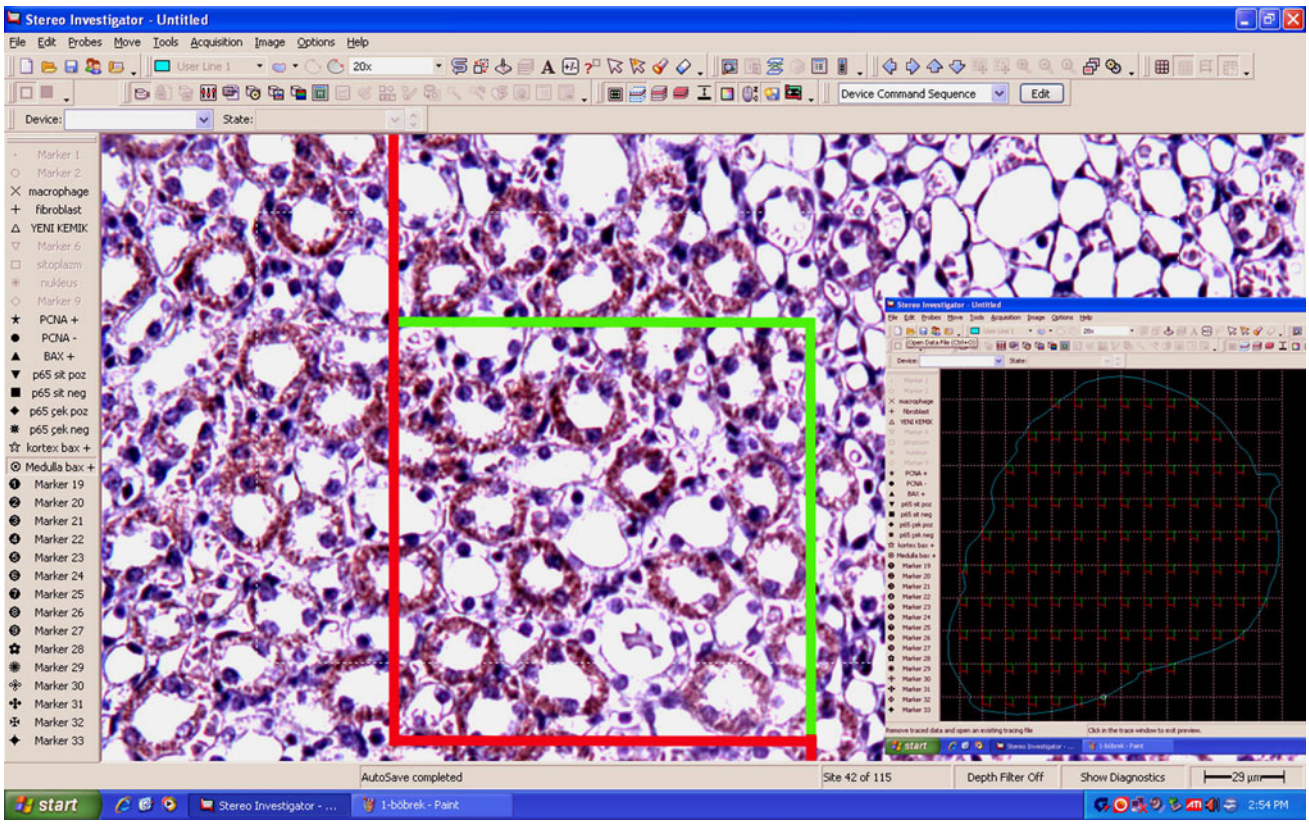


Fig. 1 Optic dissector procedure and unbiased counting frame with using stereo investigator program

Table 1 Effects of Gentamicin and *Panax ginseng* on the kidney markers and kidney weights in rat

Groups	Parameters			
	Kidney weight (g)	Creatinine (mg/dl)	Urea (mg/dl)	BUN (mg/dl)
C	0.48 ± 0.03	0.40 ± 0.12	43.55 ± 4.75	13.80 ± 4.48
GS	0.60 ± 0.04[a]	1.55 ± 0.25[a]	96.25 ± 9.50[a]	80.25 ± 5.40[a]
PG (100 mg/kg/day) + GS	0.55 ± 0.02[b]	0.95 ± 0.15[b]	72.38 ± 5.75[b]	15.50 ± 5.25[b]
PG (200 mg/kg/day) + GS	0.50 ± 0.03[b]	0.45 ± 0.20[b]	40.80 ± 7.50[b]	14.35 ± 6.50[b]

Each value represents the mean ± SEM of 8 animals

Significantly different from [a] Control group, [b] GS group ($P < 0.05$)

C Control, *PG Panax ginseng*, *GS* Gentamicin sulphate

control group, GS induced liver injury manifested by a significantly increased liver AST, ALT and GGT levels (130.25 ± 11.20 vs. 455.40 ± 18.50; 75.35 ± 7.50 vs. 220.55 ± 8.50, 125.80 ± 13.50 vs. 505.25 ± 19.52, respectively) ($P < 0.05$). This result suggests that liver function markers are elevated in the serum due to release of the enzymes from damaged liver. Conversely PG (100 and 200 mg/kg/day) treatment with GS injections caused significantly decreased above serum activities (455.40 ± 18.50 vs. 280.75 ± 15.65 and 175.40 ± 12.50, 220.55 ± 8.50 vs. 153.60 ± 6.75 and 95.45 ± 8.25, 505.25 ± 19.52 vs. 328.75 ± 13.80 and 152.50 ± 10.55, respectively) when compared with GS alone ($P < 0.05$).

Effects of GS and PG treatments on biochemical parameters

The levels of serum biochemical parameters of rats in all groups are presented in Table 3. Cholesterol and triglyceride levels were increased in the GS groups compared with the control group (130.50 ± 11.50 vs. 285.50 ± 13.25, 155.45 ± 14.45 vs. 315.60 ± 13.55, respectively)

Table 2 Effects of Gentamicin and *Panax ginseng* on hepatic markers in rat

Groups	Parameters		
	AST (U/L)	ALT (U/L)	GGT (U/L)
C	130.25 ± 11.20	75.35 ± 7.50	125.80 ± 13.50
GS	455.40 ± 18.50[a]	220.55 ± 8.50[a]	505.25 ± 19.52[a]
PG (100 mg/kg/day) + GS	280.75 ± 15.65[b]	153.60 ± 6.75[b]	328.75 ± 13.80[b]
PG (200 mg/kg/day) + GS	175.40 ± 12.50[b]	95.45 ± 8.25[b]	152.50 ± 10.55[b]

Each value represents the mean ± SEM of 8 animals

Significantly different from [a] Control group, [b] GS group ($P < 0.05$)

C Control, *PG Panax ginseng*, *GS* Gentamicin sulphate

Table 3 Effects of Gentamicin and *Panax ginseng* on serum biochemical parameters in rat

Groups	Parameters					
	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Glucose (mg/dl)	Total protein (g/dl)	Calcium (mg/dl)	Phosphorus (g/dl)
C	130.50 ± 11.50	155.45 ± 14.45	98.70 ± 10.50	52.50 ± 5.10	12.50 ± 4.50	8.25 ± 1.20
GS	285.50 ± 13.25[a]	315.60 ± 13.55[a]	105.60 ± 9.80	94.75 ± 6.50[a]	12.80 ± 3.75	7.80 ± 1.75
PG (100 mg/kg/day) + GS	205.10 ± 14.50[a]	250.50 ± 12.60[b]	102.30 ± 9.50	75.50 ± 7.20[b]	13.10 ± 5.30	8.40 ± 0.85
PG (200 mg/kg/day) + GS	165.50 ± 10.75[a]	185.70 ± 13.45[b]	100.50 ± 8.50	55.80 ± 5.40[b]	13.35 ± 4.85	8.75 ± 1.15

Each value represents the mean ± SEM of 8 animals. Significantly different from [a] Control group, [b] GS group ($P < 0.05$)

C Control, *PG Panax ginseng*, *GS* Gentamicin sulphate

($P < 0.05$). There were decreases in cholesterol and triglyceride levels in the PG (100 and 200 mg/kg/day) treatment with GS injection groups compared with the GS treated groups (285.50 ± 13.25 vs. 205.10 ± 14.50 and 165.50 ± 10.75, 315.60 ± 13.55 vs. 250.50 ± 12.60 and 185.70 ± 13.45 respectively) ($P < 0.05$).

A significant change was not determined in glucose, calcium and phosphorus levels of control and all treated groups ($P > 0.05$). However, a noticeable increase in protein levels of rats given alone GS was observed compared with control groups (52.50 ± 5.10 vs. 94.75 ± 6.50, respectively) ($P < 0.05$). But, this increase was reduced in pretreated with PG (100 and 200 mg/kg/day) compared with alone GS treated group (94.75 ± 6.50 vs. 75.50 ± 7.20 and 55.80 ± 5.40, respectively) ($P < 0.05$).

Immunohistochemical and stereological results

The Bax and Bcl-2 stereological analyses results are seen in Table 4, and Figs. 2 and 3. To investigate the apoptotic effects of PG's in GS treated rats kidneys, we determined Bax and Bcl-2 expression of both cortex and medulla. In the stereological investigations, the area of Bax positive intensity in both cortex and medulla of kidney was increased, conversely Bcl-2 activity was decreased in cortex while increased in medulla of GS treated group compared with control group. In addition, the Bax activity in both area of kidney was decreased; conversely Bcl-2 activity was decreased in cortex while increased in medulla of PG (100 and 200 mg/kg/day) groups compared with GS group. The immunohistochemical results show that low-level of apoptotic activity was seen in PG (100 and 200 mg/kg/day) treated and C groups when compared with GS treated group which apoptotic activity were increased.

Discussion

The primary antibiotics related to acute renal failure in humans and animals include aminoglycosides, beta lactams, sulfonamides, fluoroquinolones, tetracylines, etc. Researches indicate that aminoglycosides are well known to cause serious nephrotoxicity; therefore their clinical uses are limited. However, gentamicin is one of the most commonly prescribed in aminoglycosides, and its toxicity has been reported by previous studies in humans, dogs and rats. The reduction of nephrotoxicity would enhance its clinical value (Humes 1988; Walker and Duggin 1988).

The exact mechanisms by which GS induced nephrotoxicity are is not certain. However, there are many studies

reported that the specificity of GS renal toxicity may be related to its accumulation in the proximal tubules, which results in the loss of its brush border integrity (Bledsoe et al. 2006). Some authors reported that GS cause iron release from kidney cortical mitochondria and acts as iron chelators, the formed iron-drug complexes being potent catalysts of free-radical formation (Song et al. 1997; Priuska et al. 1998). Also numerous studies both in vitro and in vivo have demonstrated that GS cause reactive oxygen metabolites, particularly of H_2O_2 and O_2^-, and those have been implicated as mediators of the renal damage by inducing mesangial cells contraction and altering the filtration surface, these modifications leading to the decrease of the glomerular filtration rate (Weinberg and Humes 1980; Martínez-Salgado et al. 2002). The onset of renal injury with insufficiency induced by GS occurs after 6–14 days' treatments between of 80 and 150 mg/kg (Ben Ismail et al. 1994). In our study, GS was injected at the dose of 100 mg/kg by intraperitoneally for 10 successive days, which is well known to cause significant nephrotoxicity in rats.

In the present study, GS treatment caused marked nephrotoxicity as evidenced by the significant increase in serum urea, creatinine and BUN levels. This damage could be due to the production of reactive oxygen species by renal cortical mitochondria and involvement of oxidative stress to renal toxicity caused by GS treatment. The similar information's have been reported in previous studies where they have suggested the kidney dysfunction caused by gentamicin oxidative stress (Karahan et al. 2005; Ajami et al. 2010). PG treatment significantly decreased the elevated levels of urea, creatinine and BUN. The mechanism by which PG prevents GS-induced nephrotoxicity is not known. However the phenolic acids, flavonoids and saponins are present in the PG which may have improved the kidney functions through different antioxidant properties such as free radical scavenging activity or inhibit the formation of oxidised product. There are many studies have reported that those components of PG have been shown to have some supportive effects including antioxidant, anti-inflammatory, and anticancer effects (Kitts and Hu 2000). On the other hand similar results have been reported that several natural or synthetic antioxidants have been used successfully to the treatment of kidney damages which seems to be related to their antioxidant potentials. For example, administrations of lycopene (Karahan et al. 2005), resveratrol (Silan et al. 2007), green tea extract (Khan et al. 2009), taurine (Erdem et al. 2000), and caffeic acid phenethyl ester (Vardi et al. 2005) etc., ameliorate successfully GS-induced nephropathy.

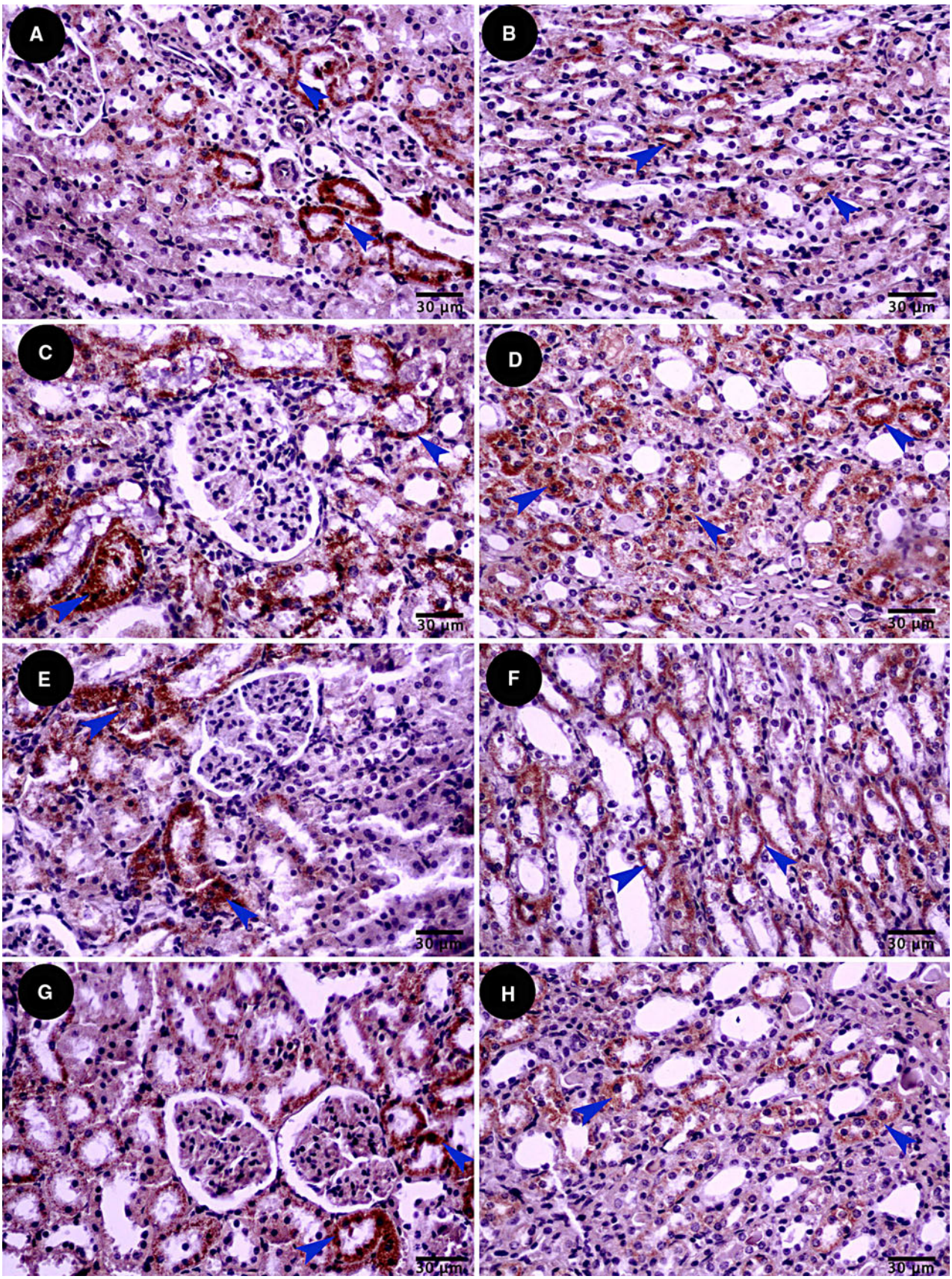
Also treatment of GS causes significant increase in the serum level of hepatic markers such as AST, ALT and GGT activities, as compared to respective control indicating hepatic dysfunction. In the present study, showed that serum AST, ALT, GGT, triglyceride, cholesterol (the lipid metabolism of liver) and total protein levels were greatly increased in GS treated rats in compare with C group. The increased of hepatic markers have been demonstrated to the hepatic damage, because these enzymes are place in cytoplasmic area of the cell and are release into blood in case of changing the permeability of hepatocyte membranes (Recknagel et al. 1991). Khan et al. (2011) stated that the gentamicin induced the increase of serum AST and ALT levels which source from cell membrane and mitochondrial damages in hepatocytes. There are some authors' reported that these enzymes activities were significantly elevated after GS treatment (Lietz and Brya 1990; Noorani et al. 2011). On the other hand, treatment with PG was found to significant suppressed an increase in serum AST, ALT and GGT activities, and triglyceride and cholesterol levels induced by GS in rats. These findings indicate that PG has nephroprotective as well as hepatoprotective effect. Already there are many authors declared that ginsenoside

Fig. 2 Bax immunoreactivity in cortex and medulla of kidney. **a** and **b** Control group, **c** and **d** GS group, **e** and **f**: PG (100 mg/kg/day) + GS group, G and H: PG (200 mg/kg/day) + GS group. Bax immunopositive cells (*arrow heads*). Streptavidin–biotin peroxidase stain, bar 30 μm

Table 4 The stereological analysis of Bax and Bcl-2 reactivity in kidney cortex and medulla

Groups	Kidney cortex*		Kidney medulla*	
	Bax	Bcl-2	Bax	Bcl-2
C	0.00134	0.00432	0.00109	0.00212
GS	0.00347[a]	0.00234[a]	0.00690[a]	0.00316[a]
PG (100 mg/kg/day) + GS	0.00147[b]	0.00211[b]	0.00192[bc]	0.00393[a]
PG (200 mg/kg/day) + GS	0.00145[b]	0.00238[b]	0.00132[b]	0.00349[a]

Each value represents the mean of 8 animals. Significantly different from [a] and [c] Control group, [b] GS group ($P < 0.05$). * Positive cells of counting frame/μm^2 area. *C* Control, PG *Panax ginseng*, *GS* Gentamicin sulphate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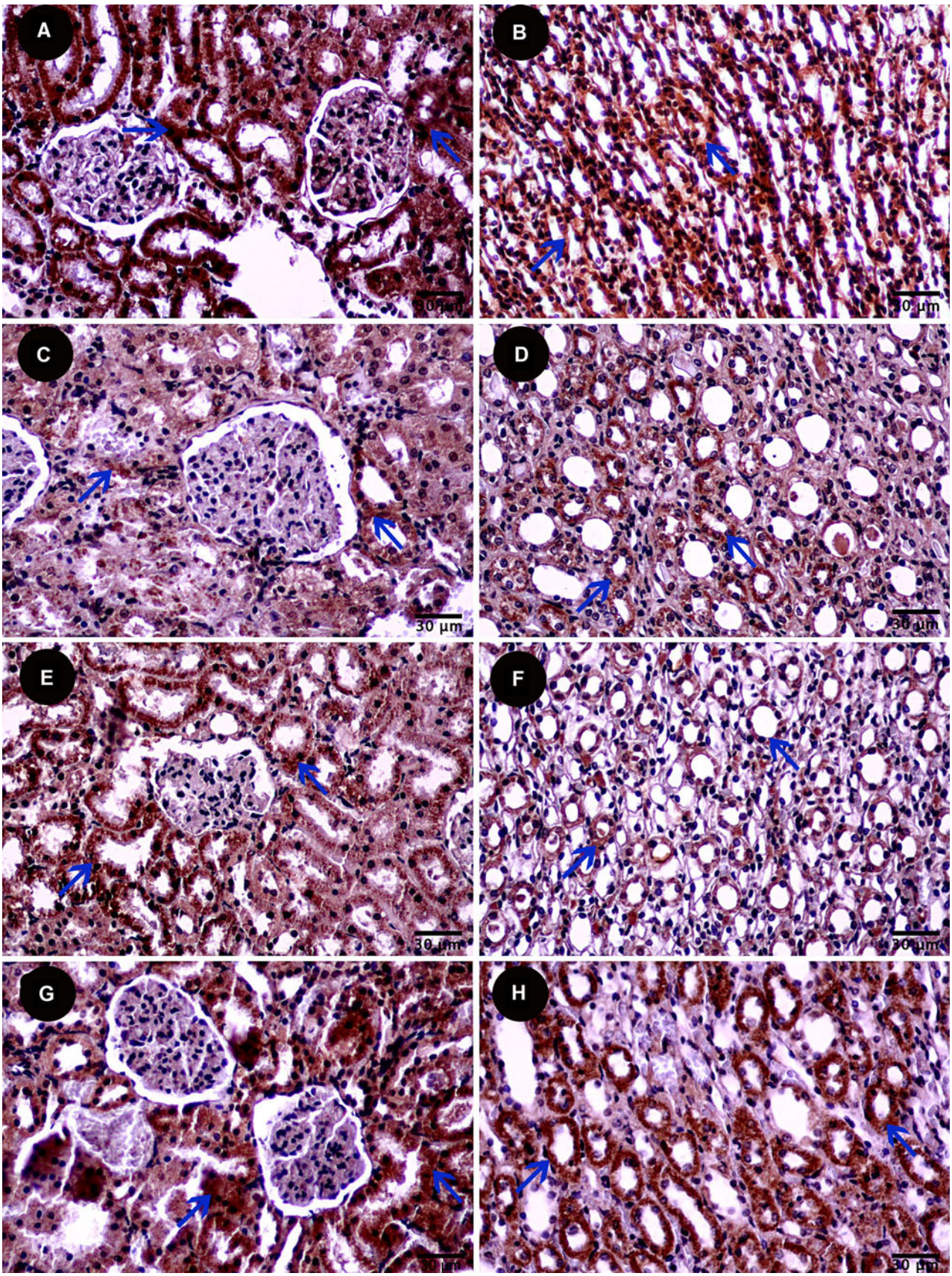
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◀ **Fig. 3** Bcl-2 immunoreactivity in cortex and medulla of kidney. **a** and **b** Control group, **c** and **d** GS group, **e** and **f** PG (100 mg/kg/day) + GS group, **g** and **h** PG (200 mg/kg/day) + GS group. Bcl-2 immunopositive cells (*arrows*). Streptavidin–biotin peroxidase stain, bar 30 μm

compounds may be responsible for its hepatoprotective effect by scavenge and destroy lipit peroxyl radicals and reactive oxygen species (Kitts and Hu 2000). Also recent studies have provided a great support for evidencing the protective effects of ginseng on kidney and liver damage (Park et al. 2005; Karadeniz et al. 2009a, b). The lipid lowering mechanisms of ginseng are mainly unknown, but recent researches informed that ginseng sapogenins has a strong inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferease in vitro, which is responsible for acylation reactions in liver (Kwon et al. 1999). However, there are recent researches reported that ginseng may have a supportive effect like that antiatherosclerotic agent by diminishing high level of serum cholesterol (Liu et al. 2010; Li et al. 2011). Also, Dixit et al. (1991) stated that of ginseng treatments markedly reduced levels of serum triglycerides and cholesterol in hyperlipidemic monkeys Also, Hwang et al. (2008) informed that the administration of ginseng saponins decreased the serum cholesterol level in rabbits which feeding high cholesterol diet. This result indicates that ginseng or sapogenins can be affecting the pathway of cholesterol biosynthesis.

We also examined the structural changes by stereological and immunohistochemical methods. In our study, analysis of apoptotic activity showed that GS treatment induced the apoptosis in both cortex and medulla of kidney tissues. Conversely, PG treatment were also decreased apoptotic activity in GS treated rat kidneys. The present results clearly show that gentamicin treatment triggers the apoptosis in kidney tissues. The Bcl-2 is known as anti-apoptotic protein and regulates the mitochondrial cytochrome *c*. The Bcl-2 proteins bind to outer membrane of mitochondria and block cytochrome *c* activation (Datta et al. 1997; Yang et al. 1997). In this study, gentamicin decreased the Bcl-2 expression and increased the Bax protein activation. Explanation of this event is that gentamicin modulates membrane enzyme activities and changes in membrane permeability so, increases in cytosolic Ca^{+} concentration, and to cause alteration of the pericellular membranes and mitochondrial membranes (Lang and Liu 1997; Jutila et al. 1998). These changes can cause the apoptotic activation in kidney. The Bax protein is known as pro-apoptotic proteins and overexpression of bax has been shown to upset the anti-apoptotic effects of bcl-2 and bcl-XL (Gross et al. 1999). These effects of Bax protein can cause increases in apoptotic activity of rat kidney, due to gentamycin treatment.

Panax ginseng-administrated rats were undergoing a decrease in apoptosis related to GS treated rats. We investigated the effects of PG on the expression of the Bax protein by immunohistochemical methods. The results showed that the levels of Bax protein in immune positivity of PG administrated kidney decreased compared to GS treated kidney tissues. Thus, these results showed that expression of the Bax proteins can be differently regulated by administration of PG. *Panax ginseng* also has an important role of the regulation of apoptosis through the expression pattern of certain key genes (Bcl-2, Bcl-xL). Moreover, some studies reported antiapoptotic effect of PG, which may be attributed to promoted expression of Bcl-2 and in this way, may cause to decrease in expression of Bax or inhibited activation of caspase-3 (Chen et al. 2002; Kim et al. 2003). Our results also show that PG inhibits GS-induced Bax activation in kidney tissues. Therefore, the inhibition of Bax activation is involved in the anti-apoptotic effect of PG in rat kidney cortex and medulla.

In conclusion, we found that PG enhanced the biochemical parameters and demonstrated a protective effect against GS-induced kidney and liver damages. Also, we showed that PG has a nephroprotective effect against apoptosis in the kidney of GS-treated rats. We suggest that PG may be used to protect against toxic effects of GS and other chemical agents in kidney and liver.

Conflict of interest The authors declare that there are no conflicts of interest.

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