Int Urol Nephrol (2010) 42:579–587
DOI 10.1007/s11255-009-9645-7

UROLOGY - ORIGINAL PAPER

Rosiglitazone, peroxisome proliferator receptor-gamma agonist, ameliorates gentamicin-induced nephrotoxicity in rats

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Received: 24 October 2008 / Accepted: 9 September 2009 / Published online: 25 September 2009

Abstract Nephrotoxicity is a major complication of gentamicin (GEN), which is widely used in the treatment of severe gram-negative infections. Reactive oxygen spaces (ROS) are important mediators of gentamicin-induced nephrotoxicity. Peroxisome proliferator-activated receptors (PPARs) have different activities including antioxidant properties. This study was performed to investigate the protective role of PPAR-γ agonist against GEN-induced nephrotoxicity. Male Wistar Albino rats were randomly divided into the following four groups, each of which consisted of six animals: (1) control; (2) intraperitoneally injected with GEN for 14 consecutive days (100 mg/kg/day); (3) treatment with rosiglitazone (RSG) via nasogastric gavage (10 mg/kg/daily for 14 days); (4) treatment with GEN + RSG combination for 14 day. Rats were decapitated on the 15th day and kidneys were removed. Urine was collected for every 24 h for the determination of daily urine volume. Urea, creatinine, Na^+ and K^+ levels were measured in blood. Malondialdehyde (MDA), reduced glutathion (GSH), and nitric oxide (NO) levels along with glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) activities were determined in the renal tissue. Changes in body weight were recorded. GEN treatment was found to cause nephrotoxicity as evidenced by elevation of serum urea and creatinine levels. Renal impairment was also assessed by the renal histology. The significant decrease in GSH and increases in MDA and NO levels as well as a decrease in GSH-Px, CAT, and SOD activities indicated that GEN-induced renal damage was mediated through oxidative reactions. On the other hand, RSG administration protected kidney tissue against GEN-induced and free radical-mediated oxidative renal damage in rats.

Keywords Peroxisome proliferator receptor-gamma agonist · Rosiglitazone · Gentamicin · Nephrotoxicity · Oxidative stress · Renal damage · Lipid peroxidation

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Introduction

Aminoglycoside antibiotics, including gentamicin (GEN), are widely used in the treatment of gram-negative infections. A major complication of these drugs are nephrotoxicity, and it has been estimated that approximately 10–20% of cases treated with aminoglycoside therapy are associated with renal dysfunction [1]. Despite the introduction of less nephrotoxic antibiotics against gram-negative micro-organism, it is still used because of low cost and efficacy against resistant beta-lactam positive micro-organisms [2]. It has been demonstrated that nephrotoxicity induced by GEN is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules [3]. The exact mechanisms of GEN-induced nephrotoxicity still remain unclear. However, several studies demonstrated that reactive oxygen species (ROS) may be important in GEN-induced nephrotoxicity [3, 4]. Moreover, GEN also has been shown to enhance the generation of ROS. Lipid peroxidation mediated by ROS have been suggested as a causative agent of cell death in different pathological states including various models of renal diseases [5, 6]. Besides their direct damaging effects on tissues, ROS seem to trigger the accumulation of leukocytes in the tissue involved, and thus also cause tissue injury indirectly through activated neutrophils. It has been shown that activated neutrophils secrete enzymes such as myeloperoxidase, elastase, and proteases, and liberate oxygen radicals [7]. On the other hand, in vivo and in vitro studies have shown that the scavengers of reactive oxygen metabolites are protective in GEN-induced renal failure [8, 9].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily and consist of three isoforms named PPAR-α, PPAR-β, and PPAR-γ. PPAR-γ is expressed in adipose tissue, endothelial cells, and vascular smooth muscle cells [10]. PPAR-γ receptor subtype appears to play an important role in the regulation of cellular proliferation and inflammation [11]. Rosiglitasone (RSG), a PPAR-γ receptor ligand, has been demonstrated to have antioxidant and anti-inflammatory effects [12]. It has been reported to reduce pulmonary and colonic inflammation in rats [13, 14], and to have beneficial effects in ischemia–perfusion injury, inflammation, and shock [15].

The potential protective effect of RSG in GEN-induced nephrotoxicity has not been studied to date. This study has been performed to investigate the possible role of RSG in protection against GEN-induced peroxidative damage in renal tissue by using biochemical and histopathological examinations.

Materials and methods

Chemicals

Gentamicin (GEN) was purchased from Bilim Pharmaceuticals, Istanbul, Turkey, and Rosiglitazone (RSG) was obtained from Glaxo Smith Kline, Brentford, Middlesex, United Kingdom. GEN was dissolved in saline and injected intraperitoneally. RSG was dissolved in distilled water and administered via nasogastric gavage.

Animals

Male Wistar Albino rats (200–250 g) were housed in clean plastic cages in a temperature and humidity-controlled facility with a constant 12 h light/dark cycle with free access to food and water. The use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.

Treatment and experimental design

After a quarantine period of 7 days, 24 rats were randomly divided into four groups, each consisting of six animals as follows: (1) the rats were control; (2) intraperitoneally injected with GEN 14 consecutive days (100 mg/kg/day); (3) treated with RSG via nasogastric gavage (10 mg/kg/daily for 14 days); (4) treated with GEN plus RSG for 14 day. Rats were decapitated on the 15th day and kidneys were removed. RSG was administered immediately after injection of GEN. Rats were treated for 14 days.

Sample collection and biochemical assays

Twenty-four hours after the administration of last doses of GEN and RSG, on 15th day, rats were

anesthetized by intraperitoneal injection of ketamine and sacrified. Twenty-four hour urine collections were obtained in standard metabolic cages a day before the rats were killed. Body weights of rats were recorded at the start and completion of experimental procedure. Renal cortical tissues were separated into two parts for biochemical analysis and light microscopic examination. Blood samples were also taken by cardiac puncture to assess the serum levels of urea, creatinine, Na^+ and K^+ concentrations. In frozen tissues biochemically malondialdehyde (MDA), end product of lipid peroxidation, reduced glutathion (GSH), nonenzymatic antioxidant, and total nitrite, a stable product of nitric oxide (NO), and the activity of glutathione peroxidase(GSH-Px), catalase (CAT), and superoxide dismutase (SOD) were evaluated as a means of oxidative stress.

Renal impairment was assessed by serum urea and creatinine levels, as well as by the kidney histology. Serum urea and creatinine levels were determined with an autoanalyzer (Syncron LX20, Ireland) by using commercial Becman Coulter diagnostic kits.

Kidney tissue (300 mg) was homogenized in ice-cold tamponade containing 150 mM KCL for determination of MDA. MDA levels were assayed for products of lipid peroxidation. MDA referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrofluorometer, as described previously [16].

GSH was determined by the spectrophotometric method, which was based on the use of Ellman's reagent [17].

Total nitrite (NO_x) was quantified by the Griess reaction [18] after incubating the supernatant with *Escherichia coli* nitrate reductase to convert NO_3 to NO_2. Griess reagent (1 ml 1% sulfanilamide, 0.1% naphtyl-ethylenediamine hydrochloride, and 2.5% phosphoric acid; Sigma Chemical Co., St. Louis, MO, USA) was then added to 1 ml of supernatant. The absorbance was read at 545 nm after a 30-min incubation. The absorbance was compared with the standard graph of $NaNO_2$, obtained from the reduction of $NaNO_3$ (1–100 μmol/l). The accuracy of the assay was checked in two ways; the inter- and intra-assay coefficients of variation were 7.52 and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), known amounts of nitrate were added to control plasma samples; these samples were deproteinized and reduced as above.

Glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine [19], by monitoring the oxidation of reduced NADPH at 340 nm. Enzyme units were defined as the number of micromoles of NADPH oxidized per minute and calculated using the extinction coefficient of NADPH at 340 nm (6.22×10^6/mol/cm). Results were reported as units per gram protein.

CAT activity was determined according to the method of Aebi, by monitoring the initial rate of disappearance of hydrogen peroxide at the 240 nm in a spectrophotometer [20]. Results were reported as the constant rate per second per gram of protein.

SOD activity was measured according to Sun et al. [21] by determining the reduction of nitroblue tetrazolium (NBT) by superoxide anion produced with xanthine and xanthine oxidase. Half unit of SOD is defined as the amount of protein that inhibited the rate of NBT reduction.

Histopathological examinations

Histopathological evaluation was made in kidney tissues. Paraffin embedded specimens were cut into 6-μm thickness and stained Hematoxylin–Eosin for light microscopic examination using a conventional protocol [22] (Olympus, BH-2, Tokyo, Japan). A semi-quantitative evaluation of renal tissues was accomplished by scoring the degree of severity according to previously published criteria [23]. All sections of kidney samples were examined for parietal cell hyperplasia, tubular vacuolization, and tubular necrosis. Briefly, minimum of 50 proximal tubules associated with 50 glomeruli were examined for each slide and an average score was obtained. Severity of lesion was graded from 0 to 3 according to the percentage of tubular involvement. Slides were examined and assigned for severity of changes using scores on scale of none (0), mild (1), moderate (2), and severe (3) damage, in which (0) denotes no change; grade (1) changes affecting <25% tubular damage (mild); grade (2) changes affecting 25–50% of tubules (moderate); grade (3) changes affecting >50% of tubules (severe).

Statistical analyses

Results of all groups were shown as mean values ± standard deviation (SD). Statistical analyses of

the histopathologic evaluation of the groups were carried out by the Chi-square test and biochemical data were analyzed by the one-way analysis of variance (ANOVA). The significance between two groups was determined by the Dunnett's multiple comparison test, and $P < 0.05$ was accepted as statistically significant value.

Results

No deaths or remarkable signs of external toxicity were observed in the groups of rats given GEN either alone or combination with RSG. The biochemical and histopathological results were similar for control and RSG groups, and we decided to consider them without distinction and report only the control group.

Body weight and urinary volume

All animals were weighed before and after the experiment. The difference between these weights was expressed as body weight chance. In group GEN, gentamicin caused a severe loss in body weight ($P < 0.001$ versus control), that was inhibited by RSG administration in group GEN + RSG ($P < 0.01$ versus group GEN; Table 1).

The 24 h urine volume in group GEN was significantly higher than in group control ($P < 0.01$), indicating the presence of GEN-induced polyuria, whereas in group GEN + RSG it was not different from that in group control, pointing out the protective role of RSG against acute tubular necrosis (Table 1).

Biochemical variables in plasma and tissue

Na^+ and K^+ concentrations among four groups were similar. Serum urea and creatinine levels were significantly higher in rats treated with GEN alone than rats in control and GEN + RSG groups ($P < 0.01$; Table 1). Administration of RSG to the GEN treated rats caused decrease in serum urea and creatinine levels.

The GSH level in renal tissue of only GEN-treated rats were significantly lower than those in control group ($P < 0.001$), and administration of RSG to GEN-treated rats significantly increased the level of GSH ($P < 0.001$; Table 2).

Group given GEN and RSG had significantly lower MDA and NO levels and higher GSH-Px, CAT, and SOD activity in kidney cortex tissue than those given GEN alone. A significant increase in the activities of these enzymatic antioxidants was noted in rats treated GEN + RSG (Table 2; $P < 0.01$).

Histopathologic examinations results

Histopathologic examination of kidney showed severe and extensive damage in GEN treated rats. These changes are summarized in Table 3. Figure 1a

Table 1 Effects of GEN alone and its combination with RSG on plasma urea, creatinine, Na^+, K^+, 24 h urine volume, and body weight changes levels in rats

Parameters	Control	GEN	RSG	GEN + RSG
Urea (mg/dl)	36 ± 4.5	120 ± 19.5[a]**	34.6 ± 3.9	44.3 ± 9.7[b]**
Creatinine (mg/dl)	0.48 ± 0.02	1.95 ± 0.4[a]**	0.46 ± 0.03	0.57 ± 0.11[b]**
Na^+ (mmol/l)	139.2 ± 2.6	142 ± 1.9[a#]	140.4 ± 2.1	141 ± 1.5[b#]
K^+ (mmol/l)	4.0 ± 0.3	4.3 ± 0.4[a#]	3.9 ± 0.4	4.1 ± 0.3[b#]
24 h urine volume (ml)	8.23 ± 0.64	24.48 ± 3.87[a]**	8.71 ± 0.03	9.41 ± 0.39[b]**
Body weight changes (g)	38 ± 1.23	−12 ± 6.03[a]***	42 ± 2.6	23.4 ± 1.92[b]**

Values are expressed as mean ± SD for six rats in each group

Groups: Control, GEN (Gentamicin), RSG (Rosiglitazone), and GEN + RSG (Gentamicin + Rosiglitazone)

[a] Compared with control group

[b] Compared with gentamicin group

[#] $P > 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 2 Effects of RSG on rat kidney NO, MDA, GSH levels and GSH-Px, CAT, SOD activities

Parameters	Control	GEN	RSG	GEN + RSG
NO (nmol/g wet tissue)	35.3 ± 6.1	102 ± 24.1[a]***	38.2 ± 4.7	42.5 ± 7.5[b]***
MDA (nmol/g wet tissue)	36.5 ± 6.4	97 ± 16.4[a]***	41.5 ± 11.6	46.3 ± 5.6[b]***
GSH (μmol/g wet tissue)	1.94 ± 0.4	0.60 ± 0.1[a]***	2.01 ± 0.5	1.64 ± 0.3[b]***
GSH-Px (IU/mg protein)	8.57 ± 1.6	7.3 ± 0.8[a]**	8.32 ± 1.2	8.6 ± 0.6[b]**
CAT (k/s/mg protein)	28 ± 0.6	19 ± 1.5[a]**	27 ± 0.5	27.5 ± 1.8[b]**
SOD (U/mg protein)	159 ± 1.6	117.4 ± 2.1[a]**	160 ± 1.4	135 ± 4.2[b]**

Values are expressed as mean ± SD for six rats in each group

Groups: Control, GEN (Gentamicin), RSG (Rosiglitazone) and GEN + RSG (Gentamicin + Rosiglitasone)

[a] Compared with control group

[b] Compared with gentamicin group

** $P < 0.01$

*** $P < 0.001$

Table 3 Semiquantitative analysis of tubular necrosis, tubular vacuolization, and parietal cell hyperplasia in control, GEN, RSG, and GEN + RSG treated rats

		Tubular necrosis				Tubular vacuolization				Parietal cell hyperplasia			
	n	0	1	2	3	0	1	2	3	0	1	2	3
Control	6	6	0	0	0	5	1	0	0	6	0	0	0
GEN[a]	6	0	0	5	1	0	1	4	1	0	2	3	1
RSG	6	6	0	0	0	4	1	1	0	4	2	0	0
GEN + RSG[b]	6	4	2	0	0	0	4	2	0	4	1	1	0

Score 0: no degeneration, 1: mild degeneration, 2: moderate degeneration, and 3: severe degeneration

[a] Statistical significant difference from the control group

[b] Statistical significant difference from the gentamicin treated group and $P < 0.05$

indicates a kidney section of a control rat while Fig. 1b shows representative images of kidneys of GEN treated rats which have tubular necrosis and edema (Fig. 1b).

In rats treated with GEN + RSG, despite the presence of mild tubular degeneration and epithelial vacuolization in the proximal tubules, parietal cell hyperplasia, and tubular necrosis are less severe, and glomeruli maintained a better morphology when compared with GEN group (Fig. 1c; Table 3).

Discussion

The kidneys are easily susceptible to damage from drugs because of larger perfusion and increased concentration of excreted compounds that occur in renal tubular cells during absorption and secretion. Aminoglycoside is an antibiotic whose clinical use is limited by its nephrotoxicity. GEN is a widely used aminoglycoside antibiotic and induced tubular necrosis [24]. Several reports have documented the pathogenesis of aminoglycoside-induced renal tubular cell injury such as derangement of lysosomal, mitochondrial, and plasma membrane structure [25, 26]. Furthermore, results of many studies have shown that altered concentrations of various biochemical indicators of oxidative stress in kidney tissue, are due to GEN [25, 26]. Because of the obvious responsibility of ROS in GEN-induced renal damage, several antioxidant agents have been used to prevent GEN nephrotoxicity [27]. In this study, we used RSG, which was able to increase antioxidant capacity [12].

The understanding of aminoglycoside nephrotoxicity is clinically important; such nephrotoxicity is typically associated with anoliguric acute renal

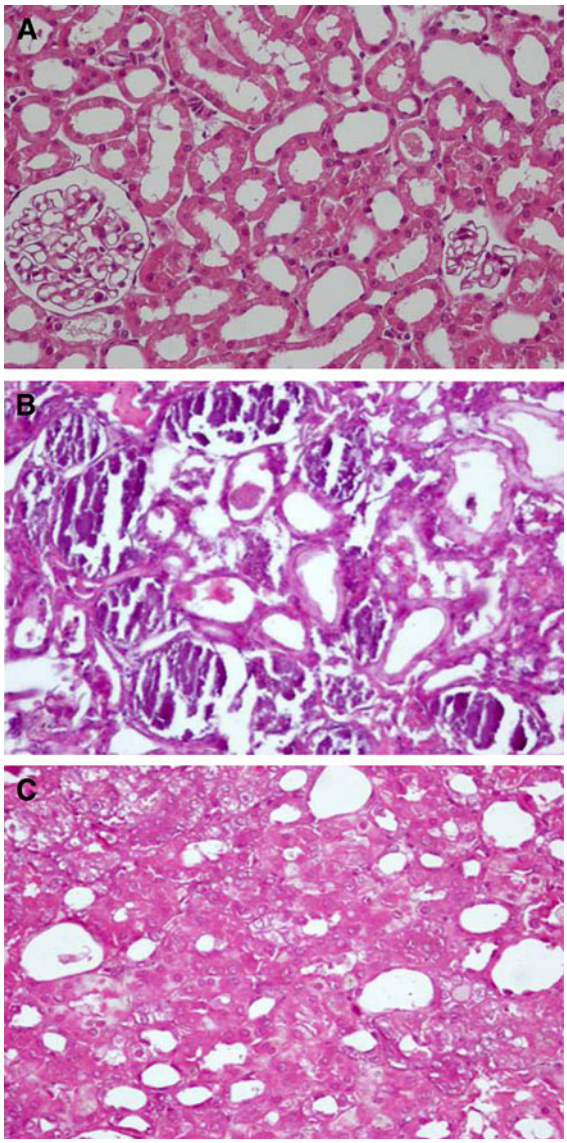

Fig. 1 Kidney morphology in: **a** a control rat, **b** a rat treated with GEN only, and **c** with GEN plus RSG (H&E ×400)

failure, i.e., azotaemia in the presence of 1–2 l/day urine output. In present study, the 24 h urine volume in the GEN group was significantly higher than in the control group, indicating the presence of GEN-induced polyuria, whereas in GEN + RSG group, it was not different from that of the control group, suggesting the protective role of RSG against acute tubular necrosis.

In the current study, increased serum urea and creatinine levels in GEN-treated rats reflect the renal damage. Administration of RSG protects the kidney function from GEN as indicated by decrease in serum urea and creatinine levels. RSG restores the renal function by preserving the structural integrity of renal cells against GEN challenge, evidenced by significantly reduced levels of serum creatinine and urea. The membrane stabilizing effect of RSG has been reported [28]. According to the recent reports, PPAR-γ agonists can improve endothelium-dependent relaxation in animals and patients with any cardiovascular disease via reduced oxidative stress [29, 30]. Therefore, RSG may also be improving renal hemodynamics indirectly through antioxidant mechanisms by inhibiting superoxide anion radicals that inactivate endothelium-derived relaxant factor. However, it has been reported that RSG improves glomerular hyperfiltration and renal endothelial dysfunction by its ability to increase bioavailability of NO [31], and RSG may be increasing renal blood flow directly through this effect. NO plays an important role in the control of renal function and long-term regulation of blood pressure [32]. NO reduces renal vascular tone in part by dilating the afferent arterioles [33]. It also increases glomerular filtration rate [34]. NO produced by nitric oxide synthases (NOS). It has been reported that RSG increases NOS expression in the kidney [35].

The blood pressure-lowering effects of PPAR-γ agonists have been reported to be accomplished, at least partially, through direct vascular effects of these agents to induce vasodilatation [36]. The beneficial effect of RSG in GEN-induced nephrotoxicity can be partly explained by the increased renal blood flow as a result of the vasodilator effect of RSG.

The peripheral vasodilatation effect of RSG may result in a transient drop in blood pressure and glomerular filtration rate. The changes may be compensated by RSG which raises the sodium reabsorption from kidneys via increasing the up-regulation of the sodium transport proteins [37].

It has been suggested that lipid peroxidation may be a contributing factor to development of GEN mediated nephropathy [35]. GEN is known to cause release of iron from renal cortical mitochondria, and also enhances the generation of the superoxide anion and hydrogen peroxide in renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of a metal catalyst, lead to the generation of toxic hydroxyl radicals which oxidize membrane lipids or bind to macromolecules,

and these reactions result in tissue injury [38]. Administration of RSG protects the cells by attenuation of lipid peroxidation, as indicated by the decreased level of MDA in kidney tissue. Thus, RSG offers protection against oxidative stress by its antioxidant properties [12].

Reduced thiol agents, such as GSH, are capable of interacting with free radicals to yield more stable elements, and are known for their ability to repair membrane lipid peroxidases [39]. Ross has reported that, cell injury and enhanced cell susceptibility to toxic chemicals are related to efflux of GSH precursors, and hence to diminished GSH biosynthesis. In this sense, GSH and other antioxidants such as vitamin C and vitamin E play a critical role in limiting the propagation of free radical reactions, which would otherwise result in extensive lipid peroxidation. In the present study, following GEN treatment, tissue GSH stores were depleted significantly, indicating the use of GSH as an antioxidant for the detoxification of toxic oxygen metabolites, while the susceptibility of the involved tissues to oxidative injury was enhanced. Administration of RSG to GEN-treated rats maintain the level of GSH to near normal levels as a result of the possible role of RSG in improving the non-enzymatic antioxidant status [12].

Superoxide dismutase is a metalloprotein and the first enzyme involved in the antioxidant functionality by lowering the steady state level of O_2^- [40]. Glutathione peroxidase catalyzes the reaction of hydroperoxide with total glutathione to form hydroperoxide [41]. Catalase is a heme protein, which catalyses the decomposition of hydroperoxide to water and oxygen, and thus protects the cell from oxidative damage by hydroperoxide and OH. There are several reports about antioxidant activity of RSG [12, 42] and it was claimed that RSG dose not revert the reduced glutathione reductase activity, but enhances the decreased xantine oxidase and superoxide dismutase activities in the gastric mucosa of ischemic rats [42]. Although xantine oxidase activity was not determined in the present study, decreases in the activities of other enzymes in GEN-treated rats reveal that lipid peroxidation and oxidative stress are elicited by GEN. The increased activity of superoxide dismutase, glutathione peroxidase and catalase in RSG-administered rats may result from the scavenging of the radicals generated by GEN-induced lipid peroxidation, thereby decreasing the utilization of these antioxidant enzymes to reduce the GEN-induced oxidative stress. This might be responsible for the increased activities of antioxidant enzymes on administration of RSG.

Recently, several studies have demonstrated that PPAR-γ agonists exhibit an anti-inflammatory effect both in vitro and in vivo. Treatment of monocytes and macrophages with high concentrations of PPAR-γ agoinsts reduces secretion of inflammatory cytokines and inhibit macrophage activation. Especially RSG has been observed to reduce the release of inflammatory cytokines such as TNF-α and IL-1β [14]. It has been recently shown that RSG treatment may have a synergistic anti-tumor activity with a decrease in nephrotoxicity through the inhibition of inflammatory cytokine release, such as TNF-α [43]. The nephroprotective effect of RSG in GEN-induced acute tubular necrosis can be partly explained by this mechanism.

The histopathologic examination of kidneys showed severe and extensive damage in GEN-treated rats which have tubular necrosis and edema. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by GEN. All these changes were histopathologically reduced in rats treated with GEN and RSG.

In conclusion, the results strongly suggest that RSG has a protective effect on GEN-induced and free radical-mediated oxidative renal damage in rats. The reduction of lipid peroxidation and elevation of antioxidant status caused by RSG, may be responsible for the protection against GEN-induced renal damage. Therefore, we found RSG as helpful in the protection of GEN-induced oxidative damage in renal tissues.

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