Journal of Trace Elements in Medicine and Biology 54 (2019) 69–78

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Toxicology

Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats

Cuneyt Caglayan[a,*], Fatih Mehmet Kandemir[b], Serkan Yildirim[c], Sefa Kucukler[b], Gizem Eser[c]

[a] Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, Bingol, Turkey
[b] Department of Biochemistry, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey
[c] Department of Pathology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey

ARTICLE INFO

Keywords:
Apoptosis
Inflammation
Mercuric chloride
Nephrotoxicity
Oxidative stress
Rutin

ABSTRACT

Objective: Mercury is a dangerous industrial and environmental pollutant which induces severe damage in diverse organs in animal and humans. The aim of this study was to investigate the protective effect of rutin (50 and 100 mg/kg body weight) against mercuric chloride ($HgCl_2$) (1.23 mg/kg b.w.) toxicity in rats.

Methods: The experiment was carried out in male Sprague Dawley rats (n = 35) which was divided into five groups as follow: control, rutin-100, $HgCl_2$, $HgCl_2$ + rutin-50 and $HgCl_2$ + rutin-100.

Results: The results showed that $HgCl_2$ caused a marked increase in the malondialdehyde (MDA) level and significantly decreased antioxidant enzyme activities ($p < 0.05$). $HgCl_2$ also provoked inflammatory responses by elevating the levels of tumor necrosis factor-α (TNF-α), B-cell lymphoma-3 (Bcl-3), interleukin-1β (IL-1β), nuclear factor kappa B (NF-κB), interleukin-33 (IL-33), and activities of mitogen-activated protein kinase 14 (MAPK 14) and myeloperoxidase (MPO) ($p < 0.05$). $HgCl_2$ also prompted the apoptotic pathway by increasing the levels of Bcl-2 associated X protein (Bax) and p53, expression of terminal deoxynucleotidyl transferase dUNT nick end labeling (TUNEL) and cysteine aspartate specific protease-3 (caspase-3). $HgCl_2$ changed histological integrity of kidney and expression of 8-hydroxy-2′-deoxyguanosine (8–OHdG) while caused a decrease in aquaporin 1 (AQP1) water channel protein level. In contrast to this, rutin significantly decreased oxidative stress, apoptosis, inflammation and histopathological alterations while increased AQP1 levels in kidney tissues ($p < 0.05$).

Conclusion: The present study indicated that rutin has a nephroprotective effect due to its anti-inflammatory, antioxidant and antiapoptotic properties.

1. Introduction

Mercury is one of the most hazardous environmental and industrial pollutants found in various chemical forms such as elemental, organic and inorganic mercury. [1,2]. Many inorganic compounds of mercury are used in agriculture (fungicides, pesticides and disinfectants) and industrial manufacture (batteries, fluorescent lamps, thermometers, thermostats) [3]. The central nervous system, gastrointestinal system, kidney and liver are the main target sites of mercury toxicity [4,5]. In addition, symptoms such as headache, impaired coordination, tremor, diarrhea, abdominal cramps, dermatitis, proteinuria, polyneuropathy and hepatic dysfunction occur as a result of mercury toxicity [3]. Mercury chloride ($HgCl_2$) is a potent nephrotoxic agent that causes acute tubular necrosis, nephritic syndrome or immunologic glomerulonephritis [5,6]. Although some studies have reported oxidative stress as a significant molecular mechanism for nephrotoxicity, the mechanism of $HgCl_2$-induced kidney injury has not been understood in detail [7,8]. The increase in oxidative stress is possibly resulted in the depletion of cellular cysteine thiols, decrease of antioxidant enzyme activity, reduction in ATP content, and production of reactive oxygen species (ROS) [6,9–12].

A specific concern about mercury exposure in people is the need for efficient treatment to cope with poisoning [6]. Plant products and their active components are the sources of natural antioxidants that can protect tissues and organs from oxidative stress and thus play an significant role in metal detoxification [13]. Flavonoids are natural polyphenolic compounds that are ubiquitous in vegetables, fruits and herbal dietary supplements [14,15]. They have antioxidant, anti-autophagic, anti-inflammatoric, anti-diabetic, anticholinergic and anti-apoptotic effects [16–20]. Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is naturally occurring

* Corresponding author at: Bingol University, Faculty of Veterinary Medicine, Department of Biochemistry, 12000, Bingol, Turkey.
E-mail address: ccaglayan@bingol.edu.tr (C. Caglayan).

https://doi.org/10.1016/j.jtemb.2019.04.007
Received 28 January 2019; Received in revised form 11 April 2019; Accepted 17 April 2019

Table 1
The effects of rutin on biochemical parameters in $HgCl_2$-induced nephrotoxicity.

Parameters	Control	Rutin-100	$HgCl_2$	$HgCl_2$ + Rutin-50	$HgCl_2$ + Rutin-100
Urea (mg/dL)	4.28 ± 0.17[a]	4.18 ± 0.12[a]	30.61 ± 1.24[d]	13.28 ± 1.47[c]	10.25 ± 1.33[b]
Creatinine (mg/dL)	0.60 ± 0.043[a]	0.57 ± 0.036[a]	3.50 ± 0.098[d]	1.52 ± 0.064[c]	1.08 ± 0.099[b]
MDA (nmol/g tissue)	33.78 ± 2.05[a]	32.17 ± 1.80[a]	116.40 ± 5.43[d]	72.97 ± 5.63[c]	50.03 ± 4.28[b]
SOD (U/g protein)	26.24 ± 1.27[a]	26.19 ± 1.52[a]	13.07 ± 0.75[c]	16.54 ± 0.84[b]	18.14 ± 0.74[b]
CAT (katal/g protein)	30.64 ± 1.15[a]	32.66 ± 3.95[a]	17.75 ± 1.01[c]	23.01 ± 1.33[b]	24.96 ± 1.00[b]
GPx (U/g protein)	33.63 ± 1.34[a]	33.65 ± 2.01[a]	13.39 ± 1.39[d]	17.14 ± 1.02[c]	22.35 ± 1.37[b]
GSH (nmol/g tissue)	3.16 ± 0.08[a]	3.25 ± 0.18[a]	1.60 ± 0.08[d]	2.28 ± 0.09[c]	2.60 ± 0.10[b]

Values are expressed as mean ± SD of seven rats in each group. Different superscripts (a–d) in the same row indicate significant difference ($p < 0.05$) among groups. (MDA; malondialdehyde, SOD; superoxide dismutase, CAT; catalase, GPx; glutathione peroxidase, GSH; glutathione).

Table 2
The effects of rutin on inflammation parameters in $HgCl_2$-induced nephrotoxicity.

Parameters	Control	Rutin-100	$HgCl_2$	$HgCl_2$ + Rutin-50	$HgCl_2$ + Rutin-100
NF-κB (ng/g tissue)	63.62 ± 1.92[a]	60.66 ± 1.75[a]	102.19 ± 4.60[d]	85.17 ± 2.73[c]	73.52 ± 2.32[b]
Bcl-3 (ng/g tissue)	7.04 ± 0.12[a]	6.77 ± 0.27[a]	10.55 ± 0.56[d]	9.19 ± 0.40[c]	8.10 ± 0.31[b]
TNF-α (ng/g tissue)	4.26 ± 0.19[a]	4.16 ± 0.10[a]	5.76 ± 0.14[c]	5.05 ± 0.14[b]	4.95 ± 1.12[b]
IL-1β (pg/g tissue)	35.67 ± 0.80[a]	34.84 ± 1.16[a]	47.22 ± 1.12[c]	40.27 ± 0.87[b]	39.22 ± 0.92[b]
IL-33 (ng/g tissue)	1.26 ± 0.05[a]	1.23 ± 0.03[a]	2.72 ± 0.04[d]	2.20 ± 0.04[c]	1.61 ± 0.04[b]
MAPK14 (ng/g tissue)	3132.11 ± 77.01[a]	3044.70 ± 82.34[a]	6120.98 ± 128.29[d]	5201.37 ± 82.46[c]	3914.71 ± 82.38[b]
MPO (ng/g tissue)	269.01 ± 10.37[a]	258.57 ± 6.57[a]	496.83 ± 12.11[c]	318.65 ± 15.20[b]	305.57 ± 9.05[b]

Values are expressed as mean ± SD of seven rats in each group. Different superscripts (a–d) in the same row indicate significant difference ($p < 0.05$) among groups. (NF-κB; nuclear factor kappa B, Bcl-3; B-cell lymphoma-3, TNF-α; tumor necrosis factor-α, IL-1β; interleukin-1β, IL-33; interleukin-33, MAPK 14; mitogen-activated protein kinase, MPO; myeloperoxidase).

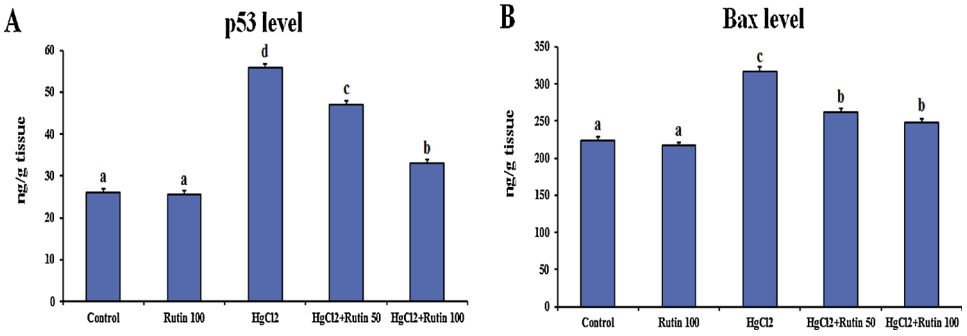

Fig. 1. (A) Effect of rutin treatment on $HgCl_2$-induced kidney p53 level in rats. (B) Effect of rutin treatment on $HgCl_2$-induced kidney Bax level in rats. Data are expressed as means ± SD. (a–d) different letters indicate statistical difference among the groups ($p < 0.05$).

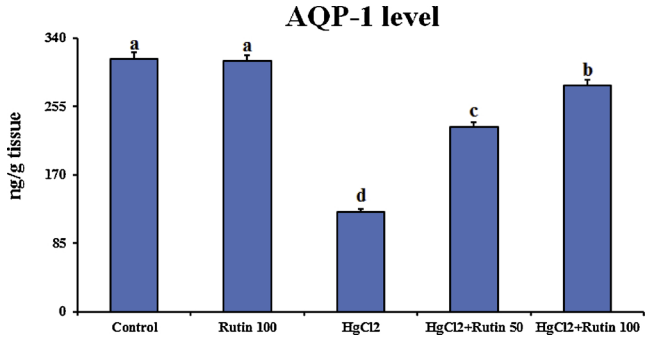

Fig. 2. Effect of rutin treatment on $HgCl_2$-induced kidney AQP1 level in rats. Data are expressed as means ± SD. (a–d) different letters indicate statistical difference among the groups ($p < 0.05$).

flavonoid glycoside that is present in passion flower, buckwheat, spinach, leaf of tomato, apples, onions and tea [21]. It is a member of bioflavonoids with antiapoptotic, antioxidant, antiallergenic, anti-inflammatory, anticarcinogenic and antiviral properties [22].

The current study was designed to investigate the protective effects of rutin against $HgCl_2$- induced nephrotoxicity. Possible molecular mechanisms of the protective effects of rutin (antioxidant, anti-apoptotic, anti-inflammatory) were investigated within the study.

2. Materials and methods

2.1. Chemicals

Mercuric chloride ($HgCl_2$, 99.5% purity), rutin (94% purity) and all other necessary reagents of analytical grade were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Animals

Thirty five male Sprague Dawley rats (250–270 g, 10–12 weeks) were purchased from Experimental Research and Application Center, Ataturk University (Erzurum, Turkey), housed under environmental conditions (25 ± 2 °C), humidity (45 ± 5%), with 12 h light-dark cycle and provided with standard pelleted rodent diet. The study protocols were approved by the Ataturk University Ethical Committee for Animal Experiments. (Permit Number: 2016-8/161).

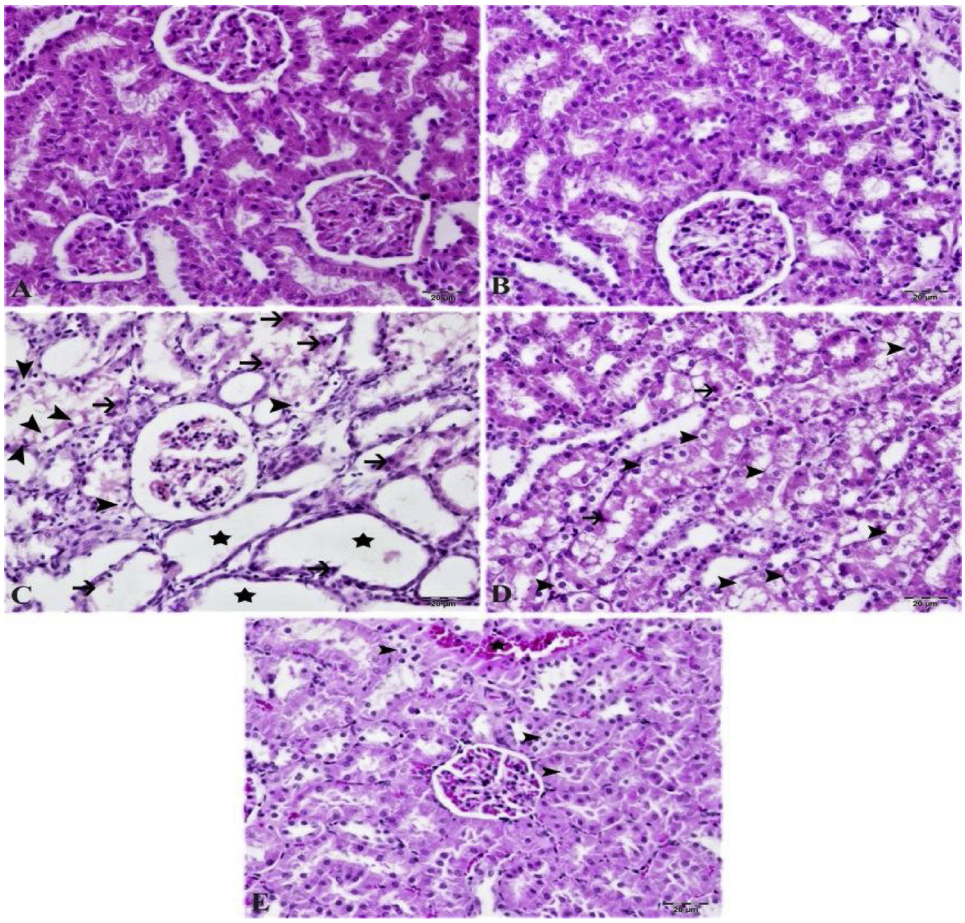

Fig. 3. (A–E) Histopathological examination of rat kidney tissue. (A) Control group: Normal histological appearance of kidney tissue. (B) Rutin 100 group: Normal histological appearance of kidney tissue. (C) $HgCl_2$ group: Degeneration (arrowheads) and necrosis (arrows) in the tubul epithelium, cystic dilatations in tubules (star) and dilatation in bowman capsule. (D) $HgCl_2$ + Rutin 50 group: Degeneration (arrowheads) and necrosis (arrows) in the tubul epithelium. (E) $HgCl_2$ + Rutin 100 grup: Slight degeneration in tubul epithelium (arrowheads) and hyperemia in interstitial vessels (star), H&E, Bar: 20 µm.

Table 3
Histopathological finding and their scores in kidney tissue.

Parameters	Control	Rutin-100	$HgCl_2$	$HgCl_2$ + Rutin-50	$HgCl_2$ + Rutin-100
Cystic dilatation in the kidney tubular	–	–	+ + +	+	–
Degeneration in the kidney tubul epithelium	–	–	+ + +	+ + +	+
Necrosis in the kidney tubul epithelium	–	–	+ + +	+ +	+
Hyperemia in interstitial vessels	–	–	+ + +	+ + +	+ +

(–) No change, (+) Mild change, (+ +) Moderate change, (+ + +) Severe change.

2.3. Experimental design

The dosage of $HgCl_2$ and rutin have been determined from previously published studies, respectively [6,22]. Rats were randomly divided into 5 groups of 7 animals each and were treated for 1 weeks as follows.

Group I (Control): Only vehicle intraperitoneal (i.p.) saline was given to rats for 7 days.

Group II (Rutin-100): the rats were administered orally rutin (100 mg/kg b.w.) for 7 days.

Group III ($HgCl_2$): the rats were administered $HgCl_2$ (1.23 mg/kg b.w.) in physiological saline i.p. for 7 days.

Group IV ($HgCl_2$ + rutin-50): the rats were administered orally rutin (50 mg/kg b.w.) 30 min after the injection of $HgCl_2$ (1.23 mg/kg b.w.) administration for 7 days.

Group V ($HgCl_2$ + rutin-100): the rats were administered orally rutin (100 mg/kg b.w.) 30 min after the injection of $HgCl_2$ (1.23 mg/kg b.w.) administration for 7 days.

Twenty-four hours after the last treatment, all rats were sacrificed under mild sevoflurane anesthesia. Blood samples were centrifuged (3000 rpm for 10 min) and serum was prepared for analysis. The kidney tissues were isolated immediately from the animals and processed for immunohistochemistry, various biochemical and histological parameters.

2.4. Serum urea and creatinine levels

The serum urea and creatinine levels were determined using a commercial kit (Diasis Diagnostic Systems, İstanbul, Turkey). The analysis was performed using an ELISA Plate Reader (Bio-Tek, Winooski, VT, USA) according to the manufacturer's instructions.

2.5. Examination of antioxidant enzyme activities and lipid peroxidation analysis

The levels of malondialdehyde (MDA), as indices of the lipid peroxidation (LPO) in kidney tissues, were measured according to the method of Placer, Cushman and Johnson [23]. Catalase (CAT) activity was performed according to method of Aebi [24]. Glutathione peroxidase (GPx) activity was measured by the method of Lawrence and Burk [25]. The superoxide dismutase (SOD) activity was assessed by the method of Sun, Oberley and Li [26]. The assay of glutathione (GSH)

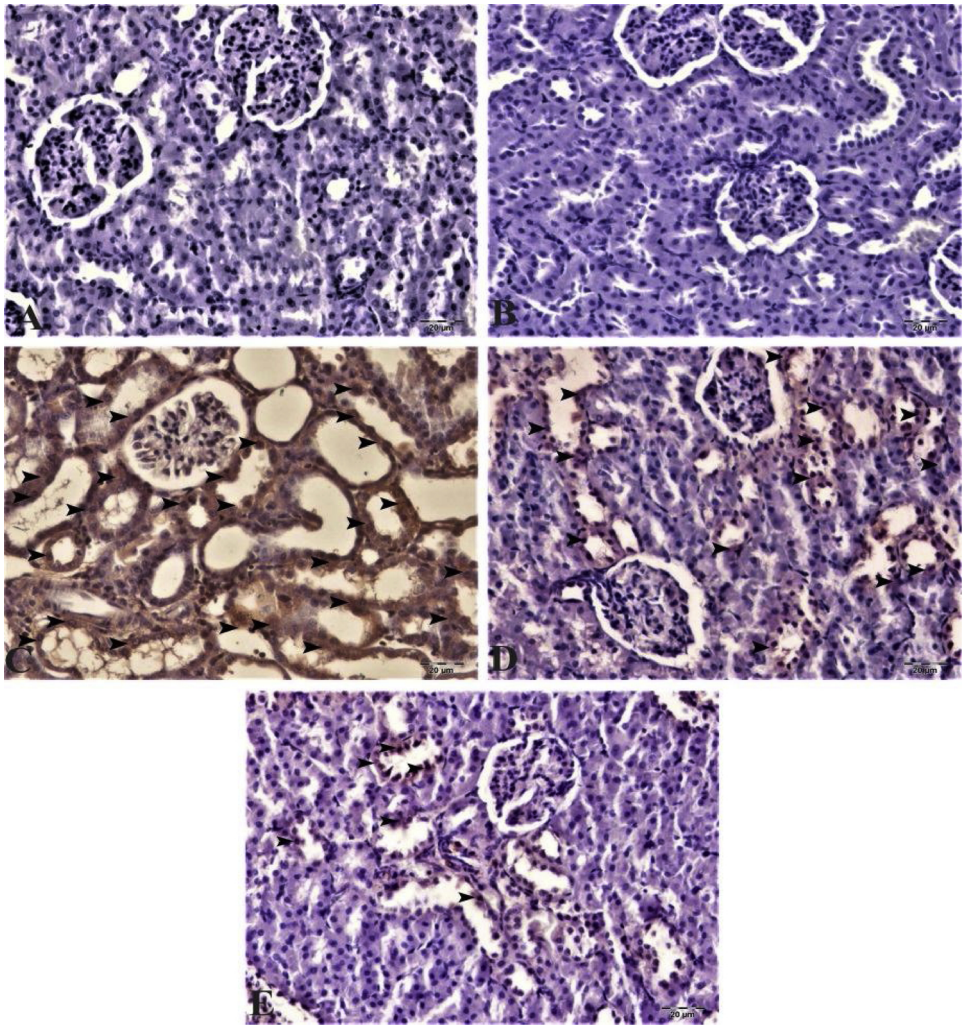

Fig. 4. (A–E) Caspase-3 expression in the rat kidney tissue (A) Control group: Negative caspase-3 expression. (B) Rutin 100 group: Negative caspase-3 expression. (C) $HgCl_2$ group: Severe caspase-3 expression (arrowhead). (D) $HgCl_2$ + Rutin 50 group: Moderate caspase-3 expression (arrowhead). (E) $HgCl_2$ + Rutin 100 grup: Mild caspase-3 expression (arrowhead), Bar: 20 μm.

level was done according to the method of Sedlak and Lindsay [27]. The protein concentration in samples was determined according to the method of Lowry, Rosebrough, Farr and Randall [28]. Biochemical analyzes were performed with ELISA Plate Reader (Bio-Tek, Winooski, VT, USA).

2.6. Tissue preparation for ELISA kits

Kidneys were rapidly homogenized in a tissue lyser device (TissueLyser II, Qiagen) using phosphate buffered saline (pH 7.4, PBS) to obtain a (1/20 wt/volume) homogenate. It was then centrifuged at 4 °C (3000 × rpm for 30 min). The upper supernatant was used for B-cell lymphoma-3(Bcl-3), nuclear factor kappa B (NF-κB), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-33 (IL-33), mitogen-activated protein kinase 14 (MAPK 14), myeloperoxidase (MPO), Bcl-2 associated X protein (Bax), p53 and aquaporin 1(AQP1) analyzes.

2.7. Measurement of inflammation markers

Levels of Bcl-3, NF-κB, IL-1β, IL-33, TNF-α, and activities of MAPK 14 and MPO in the kidney tissues was detected with a commercial rat ELISA kit (Sunred biological technology; Shangai, China) according to the manufacturer's instruction. The plates were read at 450 nm using the ELISA microplate reader (Bio-Tek, Winooski, VT, USA).

2.8. Measurement of apoptosis markers

Levels of p53 and Bax in the kidney tissues was detected with a commercial rat ELISA kit (Sunred biological technology; Shangai, China) according to the manufacturer's instruction.

2.9. Measurement of kidney AQP1 water-channel protein level

Kidney AQP1 protein levels were measured using a rat ELISA kit purchased from Sunred biological technology (Shangai, China) and was performed according to manufacturer’s instructions.

2.10. Histopathological examination

Tissue specimens taken for histopathological evaluation as a result of the necropsy performed were fixed in 10% formalin solution for 48 h. They were embedded in paraffin blocks as a result of routine tissue tracking procedures. After preparing paraffin blocks, consecutive 4 μm thick sections of the kidney tissues were cut using a microtome and stained with hematoxylin-eosin (H&E). The sections were examined under light microscopy (Leica DM 1000, Germany) for histopathological changes.

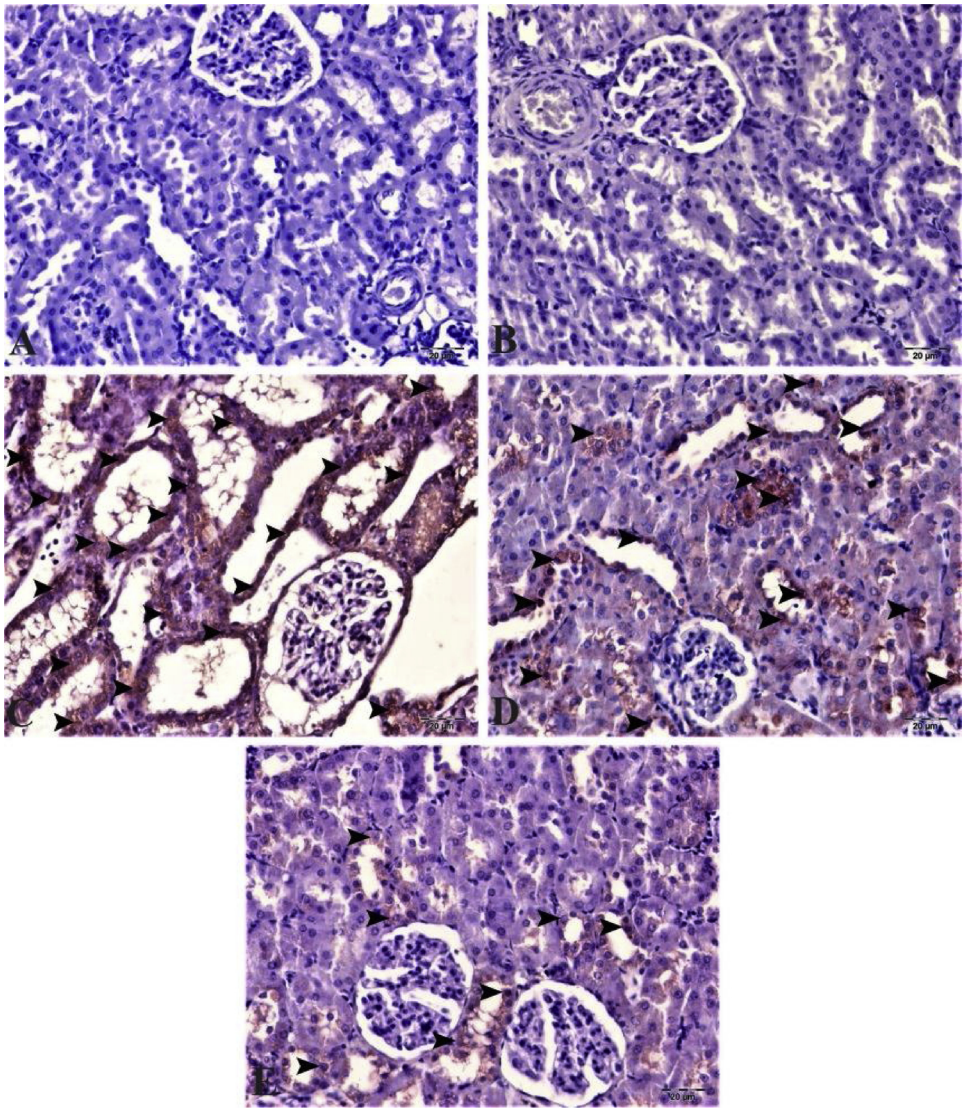

Fig. 5. (A–E) 8-OHdG expression in the rat kidney tissue (A) Control group: Negative 8-OHdG expression. (B) Rutin 100 group: Negative 8-OHdG expression. (C) $HgCl_2$ group: Severe 8-OHdG expression (arrowhead). (D) $HgCl_2$ + Rutin 50 group: Moderate 8-OHdG expression (arrowhead). (E) $HgCl_2$ + Rutin 100 grup: Mild 8-OHdG expression (arrowhead), Bar: 20 μm.

Table 4
Immunohistochemical finding and their scores in kidney tissue.

Parameters	Control	Rutin-100	$HgCl_2$	$HgCl_2$ + Rutin-50	$HgCl_2$ + Rutin-100
Caspase-3 expression	–	–	+ + +	+ +	+
8-OHdG expression	–	–	+ + +	+ +	+
TUNEL positive cell	–	–	+ + +	+ +	+

(–) No change, (+) Mild change, (+ +) Moderate change, (+ + +) Severe change.

2.11. Immunohistochemical examination

All sections put on adhesive (poly-L-Lysin) slides for immunoperoxidase analysis were passed through alcohol and xylene series. After the sections were washed with PBS, they were kept in 3% H_2O_2 for 10 min to induce endogenous peroxidase inactivation. To release the antigen from the tissues, the antigen was treated with the retrieval solution in a microwave oven for 2 × 5 min at 500 W, then was allowed to cool. Tissues were incubated for 30 min at 37 °C with Caspase 3 Antibody (Cat no: sc-65497, dilution 1/50; Santa Cruz, USA) for apoptosis detection and with 8-OHdG (Cat no: sc-66036, ready for use, thermo scientific, USA) for DNA damage detection. It was monitored according to immunohistochemical kit procedure (Abcam HRP / DAB Detection IHC kit). 3-3 'Diaminobenzidine (DAB) was used as a chromogen. Staining of ground was performed with hematoxylin. The sections were evaluated as none (−), mild (+), moderate (+ +) and severe (+ + +) according to their immune-positivity.

2.12. TUNEL method

To show DNA fragmentation in renal tissues, Terminal deoxynucleotidyl transferase-mediated-dUTP nick end labeling (TUNEL) method (In Situ Cell Death Detection Kit Roche Diagnostics; Cat. No. 11 684 817 910, Version 13.0) was selected. Staining was performed

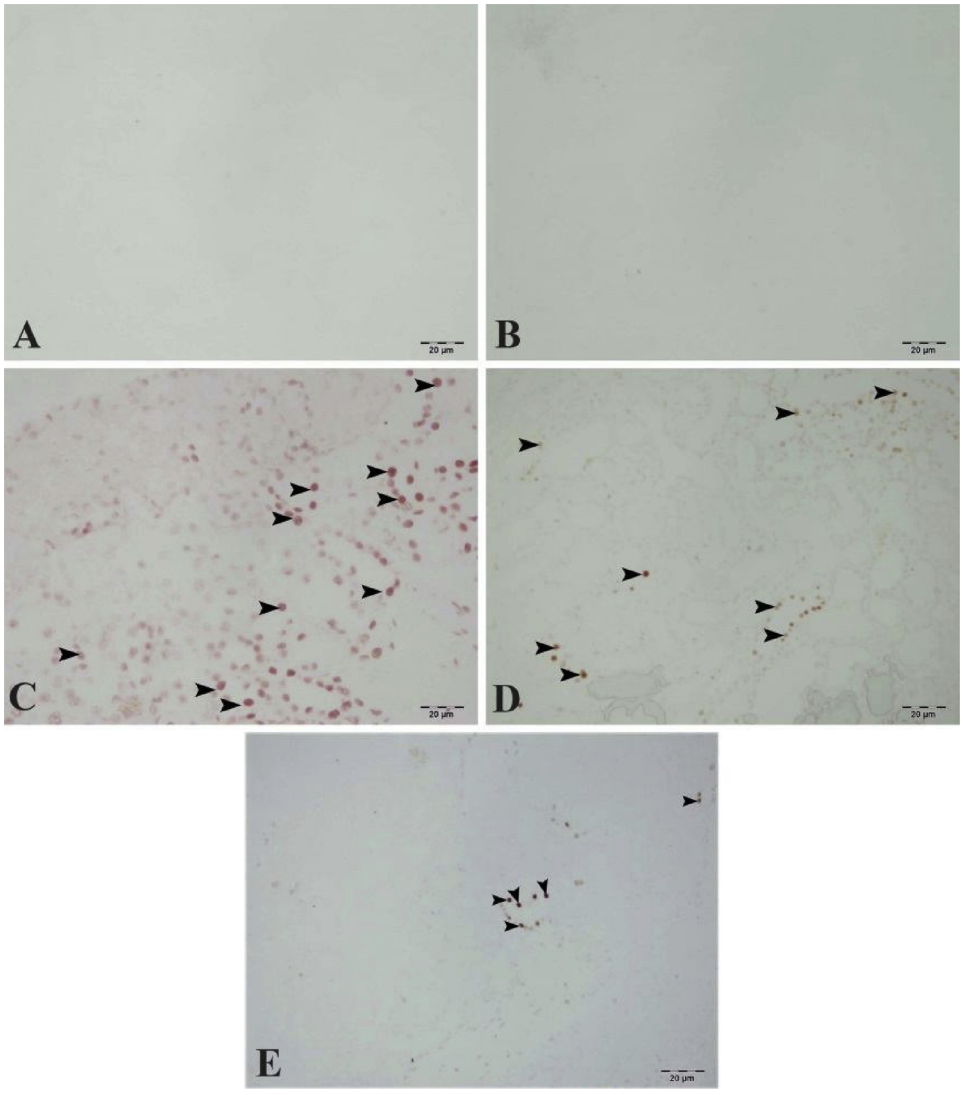

Fig. 6. (A–E) TUNEL expression in the rat kidney tissue (A) Control group: Negative TUNEL staining. (B) Rutin 100 group: Negative TUNEL staining. (C) $HgCl_2$ group: Severe positive TUNEL staining (arrowhead). (D) $HgCl_2$ + Rutin 50 group: Moderate positive TUNEL staining (arrowhead). (E) $HgCl_2$ + Rutin 100 grup: Mild positive TUNEL staining (arrowhead). TUNEL method, Bar: 20 µm.

according to the kit procedure. As chromogen, it was incubated for 5 min with 3-3' Diaminobenzidine (DAB) chromogen. It was examined with light microscope (Olympus BX51) by adhering it with Entallen. The sections were evaluated as none (−), mild 5–10 cells (+), moderate 11–20 cells (++), and severe 20 < number of cells (+++) according to the tunel positivities.

2.13. Statistical analysis

Biochemical data analyses were conducted using SPSS 20.0 software. All data were expressed as means ± standard deviations (SD). Significance levels and statistical differences were determined by using the one-way ANOVA, and Tukey's post hoc test was used to determine differences among the groups. Differences were considered significant when the $p < 0.05$.

The Kruskal-Wallis test was used for the analysis of differences of semi-quantitative obtained histopathologic for the analysis of the differences between the groups and the Mann-Whitney U test was used for the comparison of two groups. Histopathological data analyses were conducted using SPSS 13.0 software.

3. Results

3.1. Effect of treatment of rutin on Serum urea and creatinine levels

The results demonstrate that a 7.1-fold and 5.8-fold elevation in the levels of serum urea and creatinine in the $HgCl_2$-treated group when compared with the control group, respectively ($p < 0.05$). On the other hand, treatment with rutin at doses 50 and 100 mg/kg b.w. indicated a significant reduction ($p < 0.05$) in the levels of serum urea (2.30 and 2.98 fold) and creatinine (2.30 and 3.24 fold) as compared to the $HgCl_2$-treated group, respectively. There was no significant changes found in the serum creatinine and urea levels between control and the rutin only group (Table 1).

3.2. Effect of treatment of rutin on antioxidant enzyme activities

The activities of antioxidant enzymes in the kidney tissue are shown in Table 1. $HgCl_2$ toxicity diminished the activities of CAT, SOD and GPX in the kidney by 58%, 50% and 40% compared to control groups, respectively. Administration of rutin (50 and 100 mg/kg b.w.) along with $HgCl_2$ for 7 days significantly increased ($p < 0.05$) the levels of

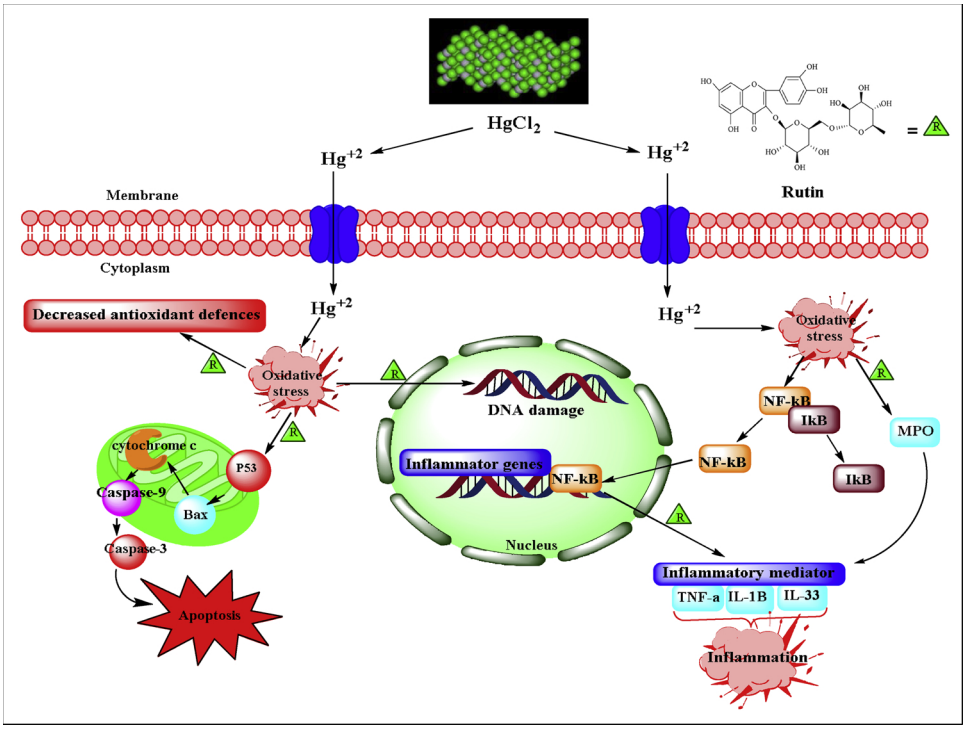

Fig. 7. Scheme summarizing the proposed protective mechanisms of rutin on $HgCl_2$-induced nephrotoxicity in rats [71].

these antioxidant enzymes as compared $HgCl_2$-treated group. The rutin only group did not exhibit any significant difference on enzymatic antioxidants in compared to the control group.

3.3. Effect of treatment of rutin on kidney MDA and GSH content

$HgCl_2$ administration caused a 3.44-fold increase in kidney MDA levels in comparison with the control group ($p < 0.05$). We observed that rutin significantly decreased MDA level in $HgCl_2$ + rutin-50 (1.60-fold) and $HgCl_2$ + rutin-100 (2.32-fold) groups as compared to the $HgCl_2$ treated group. Rutin alone did not show any significant difference in comparison with control group (Table 1).

$HgCl_2$ treatment alone decreased kidney GSH level by 50% as compared to control group. The GSH level in $HgCl_2$ + rutin-50 and $HgCl_2$ + rutin-100 groups was significantly increased in comparison with $HgCl_2$-treated group ($p < 0.05$). On the other hand, rutin only did not show any significant changes in GSH level in comparison with control group.

3.4. Effect of treatment of rutin on kidney NF-κB, TNF-α, Bcl-3, IL-33 and IL-1β levels

It was found that there was significantly elevated ($p < 0.05$) NF-κB, TNF-α, Bcl-3, IL-33 and IL-1β levels in $HgCl_2$-treated group in comparison with the control and rutin only group. However, treatment with different concentrations of rutin (50 and 100 mg/kg) significantly decreased ($p < 0.05$) these levels as compared to $HgCl_2$-group. There was no significant change in the levels of NF-κB, TNF-α, Bcl-3, IL-33 and IL-1β between the only rutin group and control group (Table 2).

3.5. Effect of treatment of rutin on kidney MAPK 14 and MPO activities

The kidney MAPK 14 and MPO activities were found to be 1.95-fold and 1.84-fold elevated in $HgCl_2$-treated group in compared to the control group, respectively. However, rutin treatment at both given doses (50 and 100 mg/kg) significantly reduction in ($p < 0.05$) these activities in comparison with $HgCl_2$-treated group (Table 2).

3.6. Effect of treatment of rutin on kidney p53 and Bax levels

$HgCl_2$ administration caused a 2.11-fold and 1.41-fold increase the p53 and Bax levels in $HgCl_2$-treated group as compared to control. Rutin treatment at both the doses (50 and 100 mg/kg) decreased $HgCl_2$ induced abnormal increase in kidney p53 and Bax levels. There was no significant difference between control group and only rutin treated group (Fig. 1A-B).

3.7. Effect of treatment of rutin on kidneyAQP1 level

The treatment with $HgCl_2$ decreased in kidney AQP1 level by 40% as compared to control group ($p < 0.05$). However there was a marked increase in AQP1 levels in case of groups treated with rutin dose dependently (Fig. 2).

3.8. Effect of rutin on the histology of the kidney

Control and rutin-100 groups were found to be in normal histological appearance (Fig. 3A–E). In the $HgCl_2$ group, cystic dilatations were seen in the kidney tubules. Severe hydropic degeneration and coagulation necrosis were seen in tubulus epithelium, while dilatation in bowman capsule were observed. In the $HgCl_2$ + rutin-50 groups, severe hydropic degeneration in tubulus epithelium, mild coagulation necrosis, and hyperemia in interstitial vessels were seen. In the $HgCl_2$ + rutin-100 groups, coagulation necrosis was not observed when hydropic degeneration was detected at very mild level. Hyperemia was detected in interstitial vessels. Histopathological findings were summarized in Table 3.

3.9. Effect of rutin on the expression of 8-OHdG and caspase-3

The expressions of 8-OHdG and caspase-3 were found to be negative in rutin-100 group as compared to the control group. At the same time, in the $HgCl_2$-exposed group, the expressions of 8-OHdG and caspase-3 were found to be strongly positive. In the $HgCl_2$ + rutin-50 and $HgCl_2$ + rutin-100 groups, the expressions of 8-OHdG and caspase-3 were

found to be moderately positive, respectively. (Figs. 4 and 5A-E). Histoptological findings were summarized in Table 4.

3.10. TUNEL findings

Given the importance of apoptosis in $HgCl_2$-induced nephrotoxicity, TUNEL assay was used to determine the extent of kidney apoptosis. As shown in Fig. 6A, there was no TUNEL-positive cell in the rutin-100 group as compared to the control group. In the $HgCl_2$ group, the TUNEL-positive cells were found at a severe level. In the $HgCl_2$ + rutin-50 and $HgCl_2$ + rutin-100 groups, the TUNEL-positive cells were found at a moderate level and mild level, respectively. (Fig. 6A–E). The TUNEL findings were summarized in Table 4.

4. Discussion

The main findings of the this study indicated that the administration of rutin attenuated oxidative stress, inflammation, apoptosis, and histopathological alterations, while it increased AQP1 permeability in the kidney against $HgCl_2$-induced nephrotoxicity.

Mercury is one of the most dangerous heavy metals that induce toxicity in brain, lung, liver, kidney [5,29], cardiorespiratory system [30] and reproductive system [31]. $HgCl_2$, one of the most toxic forms of mercury, induces oxidative stress by the production of ROS which leads to the disintegration and destabilization of the cell membrane [32]. Oxidative stress is caused by the production of ROS such as peroxides, superoxide anion radicals, and it can lead to the peroxidation of membrane lipids, protein denaturation, DNA damage and cellular injury [33,34]. It has been reported that the administration of mercury cause alterations in the mitochondrial inner membrane, resulting in the increased formation of H_2O_2 in the mitochondrial electron transport chain and depletion of mitochondrial GSH levels. [35]. Notably, mercury is able to interact with the most important thiol antioxidant, GSH, thus leading to the formation of an excretable GSH and mercury complex. This interaction decreases the levels of GSH and consequently, the reduced/oxidized glutathione ratio (GSH/GSSG), which contributes to the occurrence of oxidative stress [36,37]. Rutin (quercetin-3-rhamnosyl glucoside) works as a scavenger of ROS by donating hydrogen atoms to singlet oxygen, superoxide anion radicals, peroxy radicals and hydroxyl radicals; it also functions as a chelator of metal ions that are capable of oxidizing LPO [38]. It has been reported that rutin has a strong ability to chelate multivalent metal ions, especially calcium, zinc and iron [39]. Therefore, it is thought that the rutin decreases the toxic effects of mercury by chelating inorganic Hg^{+2} ions. Our results showed that rutin effectively reduced LPO product (MDA) level, increasing antioxidant defense system (CAT, SOD and GPx activities, and GSH level) in $HgCl_2$ treated kidney. Taken together, rutin alleviated oxidative damage in $HgCl_2$-induced kidney injury as shown in Table 1. Several studies have reported that rutin has protective effects against the nephrotoxicity induced by toxic agents such as lead acetate and potassium bromate [38,40]. In previous studies, it was also reported that rutin protects brain tissues against methylmercury-induced oxidative stress and LPO *in vitro* conditions [41,42].

Serum urea and creatinine levels are two of the most important indicators for kidney function and renal structural integrity [43]. The main target organ for mercuric salts is the kidney due to the high binding affinity between mercury and sulfhydryl groups. Mercury binds to small molecular weight thiols and metallothioneins which are abundant [44]. The interaction of mercury with protein thiol groups is thought to play an significant role in kidney toxicity induced by mercury at the cellular level [45]. In the present study, rutin resulted in a dose-dependent decrease in $HgCl_2$-induced serum urea and creatinine levels. When the kidney tissues were examined histopathologically, necrosis, degeneration, hyperemia, and cystic dilatation was observed in the tubular epithelium of $HgCl_2$ treated rats. These histopathological changes were prevented by rutin treatment in a dose-dependent manner.

Inflammation is a biological response that provides healing process for repairing damaged tissues as well as removal of harmful effects on the body [46]. NF-κB is a family of transcription factors that regulates a number of physiological processes including cell migration, cell cycle control, apoptosis and inflammation. [47]. The proper inhibition of NF-κB activation is therapeutically important because it plays a vital role in increasing levels of cytokines, such as TNF-α, IL-1β and IL-6 [48–50]. There are several studies showing mercury trigger activation of NF-κB [50,51]. Upon stimulation, inhibitor of NF-κB kinase phosphorylation undergoes inhibitor of NF-κB alpha (IκBα) ubiquitination and proteosomal degradation resulting in NF-κB liberation. Activation of NF-κB translocate into nucleus which then regulate the transcription of target genes [50]. In another study, it was reported that mercury directly induce NF-κB activation. The activation of NF-κB then upregulates the expression of many pro-inflammatory gene products such as cytokines, inducible nitric oxide synthase, and cyclooxygenase-2 [52]. However, previous research did not fully clarify the relationship between NF-κB activation and mercury exposure. In this experiment, we demonstrated that levels of Bcl-3, TNF-α, NF-κB, IL-1β and IL-33 in $HgCl_2$-induced group were increased as comparison with control group while rutin significantly showed anti-inflammatory effect by decreasing level of these markers.

Mitogen-activated protein kinases (MAPKs) have important activities as mediators of cellular responses to extracellular signals [53]. These kinase enzymes coordinately regulate the cellular responses as inflammation, apoptosis, gene expression, growth and metabolism [54]. MAPK14, known as p38α, is considered to be a central regulator of the inflammatory response in multiple cell types [55]. It has been reported that $HgCl_2$ is known to activate the MAPK pathway via generation of ROS [56]. However, little information is available on the direct effects of mercury on the MAPK14 signaling pathway. MPO, a heme protein released by leukocytes, is an enzyme that plays an important role in inflammation and oxidative stress at cellular level and it is often used as a reliable biomarker of inflammation [57]. Therefore, an increase in MPO activity in the renal cortex is considered to be a good indicator of neutrophil infiltration. [58]. In the present study, $HgCl_2$ administration significantly increased MAPK14 and MPO activities reflecting inflammatory responses. On the other hand, treatment of rutin significantly decreased activity of these enzymes.

Apoptosis, a phenomenon of programmed cell death, is a cellular process triggered by a variety of chemicals or environmental stimuli [51,59]. Several chemical and physical factors such as heavy metals, ultraviolet radiation, oxidative stress, calcium ionophores, xenobiotic agents and anoxia have been reported to cause apoptosis [43]. $HgCl_2$ causes apoptosis by cytochrome c release from mitochondrial leading to caspase-3 and 9 activities. Caspase-3, which was active as a result of cytochrome c release, causes proteolytic products of poly (ADP-ribose) polymerase (PARP) and DNA fragmentation in apoptosis [60]. Earlier studies have manifested that mercury induce apoptosis by triggering the mitochondrial pathway through the activation of caspase-3 and Bax in hepatocyte and lung cells [50,51]. In addition, Yang et al. reported recently that $HgCl_2$ induced apoptosis by increasing expression of p53, Bax, and caspase-3 in rat liver tissues [61]. TUNEL assays are also known to be used to confirm the mechanism of cell death [62]. In current study, there was increased in the number of TUNEL-positive cells and expression of caspase-3, levels of p53 and Bax protein in $HgCl_2$-treated group. However, the rutin showed a strong antiapoptotic effects by protecting tissues through decreasing number of TUNEL-positive cells and caspase-3 expressions, and Bax and p53 protein levels. Collectively, these findings demonstrated that rutin ameliorated $HgCl_2$-induced kidney damage, which is associated with the suppression of mitochondria-mediated apoptosis in rats.

Aquaporins (AQPs) are water-channel membran proteins of

approximately 30 kDa in size 13 members of the AQP isoforms (AQP0–AQP12) of which have been identified in mammals [20,63]. They have numerous important physiological and biochemical functions such as brain oedema, epithelial fluid secretion, cell migration, renal water balance and metabolism in adipocytes. AQP1 is expressed in proximal tubules and descending thin limbs of kidney. It is also found in the endothelium, including the kidney vasa recta [64]. It is known that mercury interacts with cysteine residues and inhibits most AQPs, including AQP1 [65,66]. Two hypotheses have been proposed for inhibition mechanism of AQP1 by mercury; the first is simple blockage of the channel pore by the mercury, the second is a conformational change in the interaction of the mercury atom with the cysteine residues in the aromatic constriction region (ar / R region) of AQP1 [65]. In present study, we found a marked decrease in AQP1 levels in $HgCl_2$-treated group. However, dose-dependent rutin (50 and 100 mg/kg) treatments alleviated AQP1 levels significantly.

8-OHdG is the most important and best-documented biomarker of oxidative DNA damage *in vivo* [67]. ROS may interact with DNA to produce damage such as double and single-stranded DNA breaks, nucleoside modifications and deletions. [68]. There is a linear relationship between 8-OHDG formation and ROS generation suggesting that ROS triggered the 8-OHdG formation [69]. In a previous study, it was reported that $HgCl_2$ given to human-derived liver cells (HepG2) causes oxidative DNA damage by increasing the level of 8-OHdG [70]. In this study a significant rise in expression of 8-OHdG in the $HgCl_2$-treated group was observed and this was significantly reduced after treatment with rutin.

5. Conclusion

The present study suggests that rutin has the potential to protect the kidneys from $HgCl_2$-induced damage. This protective effect against $HgCl_2$-induced nephrotoxicity may be ascribed to the antioxidant, anti-inflammatory, anti-apoptotic properties of rutin as shown in Fig.7. Collectively, our results suggest that rutin may be a promising compound for the treatment of $HgCl_2$-induced kidney toxicity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

This study was supported by Ataturk University, Foundation of Scientific Researches Projects (Project number: TDK-2017-6117).

References

[1] J.B. Nevado, R.R. Martín-Doimeadios, F.G. Bernardo, M.J. Moreno, A.M. Herculano, J. Do Nascimento, M.E. Crespo-López, Mercury in the Tapajós River basin, Brazilian amazon: a review, Environ. Int. 36 (6) (2010) 593–608.
[2] W. Crowe, P.J. Allsopp, G.E. Watson, P.J. Magee, J. Strain, D.J. Armstrong, E. Ball, E.M. McSorley, Mercury as an environmental stimulus in the development of autoimmunity–a systematic review, Autoimmun. Rev. 16 (1) (2017) 72–80.
[3] D. Joshi, D.K. Mittal, R. Kumar, A. Kumar Srivastav, S.K. Srivastav, Protective role of Curcuma longa extract and curcumin on mercuric chloride-induced nephrotoxicity in rats: evidence by histological architecture, Toxicol. Environ. Chem. 95 (9) (2013) 1581–1588.
[4] R. Agarwal, J.R. Behari, Role of selenium in mercury intoxication in mice, Ind. Health 45 (3) (2007) 388–395.
[5] A. Aslanturk, M. Uzunhisarcikli, S. Kalender, F. Demir, Sodium selenite and vitamin E in preventing mercuric chloride induced renal toxicity in rats, Food Chem. Toxicol. 70 (2014) 185–190.
[6] E. Bharathi, G. Jagadeesan, Antioxidant potential of hesperidin and ellagic acid on renal toxicity induced by mercuric chloride in rats, Biomed. Prev. Nutr. 4 (2) (2014) 131–136.
[7] J. Nikolic, G. Kocic, T. Jevtovic-Stojmenov, Effect of bioflavonoid lespeflan on xanthine oxidase activity in mercury chloride toxicity, Pharmacologyonline 3 (2006) 669–675.
[8] M.T. Boroushaki, H. Mollazadeh, A. Rajabian, K. Dolati, A. Hoseini, M. Paseban, M. Farzadnia, Protective effect of pomegranate seed oil against mercuric chloride-induced nephrotoxicity in rat, Ren. Fail. 36 (10) (2014) 1581–1586.
[9] S. Vijayaprakash, K. Langeswaran, S.G. Kumar, R. Revathy, M.P. Balasubramanian, Nephro-protective significance of kaempferol on mercuric chloride induced toxicity in Wistar albino rats, Biomed. Aging Pathol. 3 (3) (2013) 119–124.
[10] İ. Gülçin, Antioxidant and antiradical activities of L-carnitine, Life Sci. 78 (8) (2006) 803–811.
[11] T. Ak, İ. Gülçin, Antioxidant and radical scavenging properties of curcumin, Chem.-Biol. Interact. 174 (1) (2008) 27–37.
[12] I. Gülçin, Antioxidant activity of food constituents: an overview, Arch. Toxicol. 86 (3) (2012) 345–391.
[13] D. Joshi, D.K. Mittal, S. Shukla, S.K. Srivastav, V.A. Dixit, Curcuma longa Linn. extract and curcumin protect CYP 2E1 enzymatic activity against mercuric chloride-induced hepatotoxicity and oxidative stress: a protective approach, Exp. Toxicol. Pathol. 69 (6) (2017) 373–382.
[14] E. Köksal, E. Bursal, İ. Gülçin, M. Korkmaz, C. Çağlayan, A.C. Gören, S.H. Alwasel, Antioxidant activity and polyphenol content of Turkish thyme (Thymus vulgaris) monitored by liquid chromatography and tandem mass spectrometry, Int. J. Food Prop. 20 (3) (2017) 514–525.
[15] F.M. Kandemir, S. Kucukler, E. Eldutar, C. Caglayan, I. Gülçin, Chrysin protects rat kidney from paracetamol-induced oxidative stress, inflammation, apoptosis, and autophagy: a Multi-biomarker approach, Sci. Pharm. 85 (1) (2017) 4.
[16] P. Taslimi, C. Caglayan, İ. Gulcin, The impact of some natural phenolic compounds on carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes: an antidiabetic, anticholinergic, and antiepileptic study, J. Biochem. Mol. Toxicol. 31 (12) (2017) e21995.
[17] E. Eldutar, F.M. Kandemir, S. Kucukler, C. Caglayan, Restorative effects of Chrysin pretreatment on oxidant–antioxidant status, inflammatory cytokine production, and apoptotic and autophagic markers in acute paracetamol-induced hepatotoxicity in rats: an experimental and biochemical study, J. Biochem. Mol. Toxicol. 31 (11) (2017) e21960.
[18] F. Benzer, F.M. Kandemir, M. Ozkaraca, S. Kucukler, C. Caglayan, Curcumin ameliorates doxorubicin-induced cardiotoxicity by abrogation of inflammation, apoptosis, oxidative DNA damage, and protein oxidation in rats, J. Biochem. Mol. Toxicol. 32 (2) (2018) e22030.
[19] M. Kuzu, F.M. Kandemir, S. Yildirim, S. Kucukler, C. Caglayan, E. Turk, Morin attenuates doxorubicin-induced heart and brain damage by reducing oxidative stress, inflammation and apoptosis, Biomed. Pharmacother. 106 (2018) 443–453.
[20] F.M. Kandemir, S. Yildirim, S. Kucukler, C. Caglayan, A. Mahamadu, M.B. Dortbudak, Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney, Biomed. Pharmacother. 105 (2018) 981–991.
[21] E. Aksu, F. Kandemir, M. Özkaraca, A. Ömür, S. Küçükler, S. Çomaklı, Rutin ameliorates cisplatin-induced reproductive damage via suppression of oxidative stress and apoptosis in adult male rats, Andrologia 49 (1) (2017) e12593.
[22] S. Nafees, S. Rashid, N. Ali, S.K. Hasan, S. Sultana, Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway, Chem.-Biol. Interact. 231 (2015) 98–107.
[23] Z.A. Placer, L.L. Cushman, B.C. Johnson, Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems, AnBio 16 (2) (1966) 359–364.
[24] H. Aebi, 13] Catalase in vitro, Methods Enzymol. Elsevier, 1984, pp. 121–126.
[25] R.A. Lawrence, R.F. Burk, Glutathione peroxidase activity in selenium-deficient rat liver, BBRC 71 (4) (1976) 952–958.
[26] Y. Sun, L.W. Oberley, Y. Li, A simple method for clinical assay of superoxide dismutase, Clin. Chem. 34 (3) (1988) 497–500.
[27] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent, AnBio 25 (1968) 192–205.
[28] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1) (1951) 265–275.
[29] G. Şener, Ö. Sehirli, A. Tozan, A. Velioğlu-Övunç, N. Gedik, G.Z. Omurtag, Ginkgo biloba extract protects against mercury (II)-induced oxidative tissue damage in rats, Food Chem. Toxicol. 45 (4) (2007) 543–550.
[30] B.F. Azevedo, Hd.A.F. Neto, I. Stefanon, D.V. Vassallo, Acute cardiorespiratory effects of intracisternal injections of mercuric chloride, Neurotoxicology 32 (3) (2011) 350–354.
[31] G.E. El-Desoky, S.A. Bashandy, I.M. Alhazza, Z.A. Al-Othman, M.A. Aboul-Soud, K. Yusuf, Improvement of mercuric chloride-induced testis injuries and sperm quality deteriorations by Spirulina platensis in rats, PLoS One 8 (3) (2013) e59177.
[32] D. Joshi, S.K. Srivastav, S. Belemkar, V.A. Dixit, Zingiber officinale and 6-gingerol alleviate liver and kidney dysfunctions and oxidative stress induced by mercuric chloride in male rats: a protective approach, Biomed. Pharmacother. 91 (2017) 645–655.
[33] M. Kirici, C. Turk, C. Caglayan, M. Kirici, Toxic effects of copper sulphate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish capoeta umbla (heckel, 1843) tissues, Appl. Ecol. Environ. Res 15 (2017) 1685–1696.
[34] F.E. Agha, E.R. Youness, M.M. Selim, H.H. Ahmed, Nephroprotective potential of selenium and taurine against mercuric chloride induced nephropathy in rats, Ren. Fail. 36 (5) (2014) 704–716.
[35] S. Kalender, F.G. Uzun, F. Demir, M. Uzunhisarcıklı, A. Aslanturk, Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E, Food Chem. Toxicol. 55 (2013) 456–462.
[36] M. Farina, D.S. Avila, J.B.T. Da Rocha, M. Aschner, Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury, Neurochem. Int. 62 (5) (2013) 575–594.
[37] M. Farina, M. Aschner, J.B. Rocha, Oxidative stress in MeHg-induced neurotoxicity, Toxicol. Appl. Pharmacol. 256 (3) (2011) 405–417.

[38] R.A. Khan, M.R. Khan, S. Sahreen, Protective effects of rutin against potassium bromate induced nephrotoxicity in rats, BMC Complement. Altern. Med. 12 (1) (2012) 204.
[39] R.A. Khan, M.R. Khan, S. Sahreen, CCl 4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat, BMC Complement. Altern. Med. 12 (1) (2012) 178.
[40] S. Ansar, S. Hamed, H. AlGhosoon, R. AlSaedan, M. Iqbal, The protective effect of rutin against renal toxicity induced by lead acetate, Toxin Rev. 35 (1–2) (2016) 58–62.
[41] J.L. Franco, T. Posser, F. Missau, M.G. Pizzolatti, A.R. Santos, D.O. Souza, M. Aschner, J.B. Rocha, A.L. Dafre, M. Farina, Structure–activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction, Environ. Toxicol. Pharmacol. 30 (3) (2010) 272–278.
[42] C. Wagner, A.P. Vargas, D.H. Roos, A.F. Morel, M. Farina, C.W. Nogueira, M. Aschner, J.B. Rocha, Comparative study of quercetin and its two glycoside derivatives quercitrin and rutin against methylmercury (MeHg)-induced ROS production in rat brain slices, Arch. Toxicol. 84 (2) (2010) 89–97.
[43] E. Turk, F.M. Kandemir, S. Yildirim, C. Caglayan, S. Kucukler, M. Kuzu, Protective effect of Hesperidin on sodium arsenite-induced nephrotoxicity and hepatotoxicity in rats, Biol. Trace Elem. Res. (2018) 1–14.
[44] R. Agarwal, S.K. Goel, R. Chandra, J.R. Behari, Role of vitamin E in preventing acute mercury toxicity in rat, Environ. Toxicol. Pharmacol. 29 (1) (2010) 70–78.
[45] A.M. Gado, B.A. Aldahmash, Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity, Drug Des. Devel. Ther. 7 (2013) 1245.
[46] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (6) (2010) 805–820.
[47] N.J. Thornburg, R. Pathmanathan, N. Raab-Traub, Activation of nuclear factor-κB p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma, Cancer Res. 63 (23) (2003) 8293–8301.
[48] F.M. Kandemir, S. Kucukler, C. Caglayan, C. Gur, A.A. Batil, İ. Gülçin, Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties, J. Food Biochem. 41 (5) (2017) e12398.
[49] F. Benzer, F.M. Kandemir, S. Kucukler, S. Comaklı, C. Caglayan, Chemoprotective effects of curcumin on doxorubicin-induced nephrotoxicity in wistar rats: by modulating inflammatory cytokines, apoptosis, oxidative stress and oxidative DNA damage, Arch. Physiol. Biochem. (2018) 1–10.
[50] B. Liu, H. Yu, R. Baiyun, J. Lu, S. Li, Q. Bing, X. Zhang, Z. Zhang, Protective effects of dietary luteolin against mercuric chloride-induced lung injury in mice: Involvement of AKT/Nrf2 and NF-κB pathways, Food Chem. Toxicol. 113 (2018) 296–302.
[51] P.B. Pal, S. Pal, J. Das, P.C. Sil, Modulation of mercury-induced mitochondria-dependent apoptosis by glycine in hepatocytes, Amino Acids 42 (5) (2012) 1669–1683.
[52] H.-J. Park, H.-S. Youn, Mercury induces the expression of cyclooxygenase-2 and inducible nitric oxide synthase, Toxicol. Ind. Health 29 (2) (2013) 169–174.
[53] S.H. Kim, V.J. Johnson, R.P. Sharma, Mercury inhibits nitric oxide production but activates proinflammatory cytokine expression in murine macrophage: differential modulation of NF-κB and p38 MAPK signaling pathways, Nitric Oxide 7 (1) (2002) 67–74.
[54] N. Umasuthan, S. Bathige, J.K. Noh, J. Lee, Gene structure, molecular characterization and transcriptional expression of two p38 isoforms (MAPK11 and MAPK14) from rock bream (Oplegnathus fasciatus), Fish Shellfish Immunol. 47 (1) (2015) 331–343.
[55] U. Lo, V. Selvaraj, J.M. Plane, O.V. Chechneva, K. Otsu, W. Deng, p38α (MAPK14) critically regulates the immunological response and the production of specific cytokines and chemokines in astrocytes, Sci. Rep. 4 (2014) 7405.
[56] J. Lee, S.J. Lee, K.T. Lim, Preventive effects of ZPDC glycoprotein (24 kDa) on hepatotoxicity induced by mercury chloride in vitro and in vivo, Cell Biochem. Funct. 32 (6) (2014) 520–529.
[57] N. Anatoliotakis, S. Deftereos, G. Bouras, G. Giannopoulos, D. Tsounis, C. Angelidis, A. Kaoukis, C. Stefanadis, Myeloperoxidase: expressing inflammation and oxidative stress in cardiovascular disease, Curr. Top. Med. Chem. 13 (2) (2013) 115–138.
[58] B.D. Sahu, S. Tatireddy, M. Koneru, R.M. Borkar, J.M. Kumar, M. Kuncha, R. Srinivas, R. Sistla, Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: possible mechanism of nephroprotection, Toxicol. Appl. Pharmacol. 277 (1) (2014) 8–20.
[59] C. Caglayan, F.M. Kandemir, S. Yıldırım, S. Kucukler, M.A. Kılınc, Y.S. Saglam, Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female wistar rats, Biomed. Pharmacother. 102 (2018) 517–530.
[60] S. Araragi, M. Kondoh, M. Kawase, S. Saito, M. Higashimoto, M. Sato, Mercuric chloride induces apoptosis via a mitochondrial-dependent pathway in human leukemia cells, Toxicology 184 (1) (2003) 1–9.
[61] D. Yang, X. Tan, Z. Lv, B. Liu, R. Baiyun, J. Lu, Z. Zhang, Regulation of Sirt1/Nrf2/TNF-α signaling pathway by luteolin is critical to attenuate acute mercuric chloride exposure induced hepatotoxicity, Sci. Rep. 6 (2016) 37157.
[62] S. Yıldırım, Investigation by histopathological and TUNEL method of the protective effect of Resveratrol on carbon tetrachloride induced liver toxicity in rats, Atatürk Üniversitesi Veteriner Bilimleri Dergisi 12 (2) (2017) 178–186.
[63] K. Kobayashi, M. Yasui, Cellular and subcellular localization of aquaporins 1, 3, 8, and 9 in amniotic membranes during pregnancy in mice, Cell Tissue Res. 342 (2) (2010) 307–316.
[64] A.S. Verkman, M.O. Anderson, M.C. Papadopoulos, Aquaporins: important but elusive drug targets, Nat. Rev. Drug Discov. 13 (4) (2014) 259.
[65] Y. Hirano, N. Okimoto, I. Kadohira, M. Suematsu, K. Yasuoka, M. Yasui, Molecular mechanisms of how mercury inhibits water permeation through aquaporin-1: understanding by molecular dynamics simulation, BPJ 98 (8) (2010) 1512–1519.
[66] G.M. Preston, J.S. Jung, W.B. Guggino, P. Agre, The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel, J. Biol. Chem. 268 (1) (1993) 17–20.
[67] F.M. Kandemir, M. Ozkaraca, S. Küçükler, C. Caglayan, B. Hanedan, Preventive effects of hesperidin on diabetic nephropathy induced by streptozotocin via modulating TGF-β1 and oxidative DNA damage, Toxin Rev. (2017) 1–7.
[68] P. Subash, P. Gurumurthy, A. Sarasabharathi, K. Cherian, Urinary 8-OHdG: a marker of oxidative stress to DNA and total antioxidant status in essential hypertension with South Indian population, Indian J. Clin. Biochem. 25 (2) (2010) 127–132.
[69] C. Caglayan, Y. Temel, F.M. Kandemir, S. Yildirim, S. Kucukler, Naringin protects against cyclophosphamide-induced hepatotoxicity and nephrotoxicity through modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage, Environ. Sci. Pollut. Res. - Int. (2018) 1–17.
[70] G.R.M. Barcelos, J.P.F. Angeli, J.M. Serpeloni, D. Grotto, B.A. Rocha, J.K. Bastos, S. Knasmüller, F.B. Júnior, Quercetin protects human-derived liver cells against mercury-induced DNA-damage and alterations of the redox status, Mutat. Res. Toxicol. Environ. Mutagen. 726 (2) (2011) 109–115.
[71] X. Tan, B. Liu, J. Lu, S. Li, R. Baiyun, Y. Lv, Q. Lu, Z. Zhang, Dietary luteolin protects against HgCl2-induced renal injury via activation of Nrf2-mediated signaling in rat, J. Inorg. Biochem. 179 (2018) 24–31.