ORIGINAL ARTICLE

Neuroprotective effect of rutin against colistin-induced oxidative stress, inflammation and apoptosis in rat brain associated with the CREB/BDNF expressions

Hamit Çelik[1] · Fatih Mehmet Kandemir[2] · Cuneyt Caglayan[3] · Selçuk Özdemir[4] · Selim Çomaklı[5] · Sefa Kucukler[2] · Ahmet Yardım[6]

Abstract

The purpose of the current study was to examine the neuroprotective effect of rutin against colistin-induced neurotoxicity in rats. Thirty-five male Sprague Dawley rats were randomly divided into 5 groups. The control group (orally received physiological saline), the rutin group (orally administered 100 mg/kg body weight), the colistin group (i.p. administered 15 mg/kg body weight), the Col + Rut 50 group (i.p. administered 15 mg/kg body weight of colistin, and orally received 50 mg/kg body weight of rutin), the Col + Rut 100 group (i.p. administered 15 mg/kg body weight of colistin, and orally received 100 mg/kg body weight of rutin). Administration of colistin increased levels of glial fibrillary acidic protein and brain-derived neurotrophic factor and acetylcholinesterase and butyrylcholinesterase activities while decreasing level of cyclic AMP response element binding protein and extracellular signal regulated kinases 1 and 2 (ERK1/2) expressions. Colistin increased oxidative impairments as evidenced by a decrease in level of nuclear factor erythroid 2-related factor 2 (Nrf-2), glutathione, superoxide dismutase, glutathione peroxidase and catalase activities, and increased malondialdehyde content. Colistin also increased the levels of the apoptotic and inflammatoric parameters such as cysteine aspartate specific protease-3 (caspase-3), p53, B-cell lymphoma-2 (Bcl-2), nuclear factor kappa B (NF-κB), Bcl-2 associated X protein (Bax), tumor necrosis factor-α (TNF-α) and neuronal nitric oxide synthase (nNOS). Rutin treatment restored the brain function by attenuating colistin-induced oxidative stress, apoptosis, inflammation, histopathological and immunohistochemical alteration suggesting that rutin supplementation mitigated colistin-induced neurotoxicity in male rats.

Keywords Apoptosis · Colistin · Inflammation · Oxidative stress · Neurotoxicity · Rutin

✉ Fatih Mehmet Kandemir
fkandemir03@gmail.com

✉ Cuneyt Caglayan
ccaglayan@bingol.edu.tr

[1] Department of Neurology, Private Buhara Hospital, Erzurum, Turkey

[2] Department of Biochemistry, Faculty of Veterinary Medicine, Atatürk University, 25240 Erzurum, Turkey

[3] Department of Biochemistry, Faculty of Veterinary Medicine, Bingol University, 12000 Bingol, Turkey

[4] Department of Genetics, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

[5] Department of Pathology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Turkey

[6] Department of Neurosurgery, Private Buhara Hospital, Erzurum, Turkey

Introduction

Colistin (polymyxin E), a cationic polypeptide antibiotic, has been used for the treatment of infections of multidrug-resistant gram-negative bacteria such as *Klebsiella pneumonia, Acinetobacter baumannii* and *Pseudomonas aeruginosa* [1]. The main target of antimicrobial activity of colistin is the bacterial cell membrane. Colistin interacts to anionic lipopolysaccharides of Gram-negative bacteria and causes the replacement of divalent cations such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) in the membrane. As a result, it causes an increase in the bacterial cell permeability and subsequently, cell death [2, 3]. However, neurotoxicity and nephrotoxicity are still major adverse effects limiting its clinical uses [1, 4, 5]. The neurological symptoms such as hallucination, dizziness, confusion, seizures, vertigo and visual disturbances were recorded in colistin-treated

patients [6, 7]. In previous studies, colistin has been reported to induce axonal and neuronal degeneration in brain and sciatic nerves when intravenously administrated in mice at a dose of 15 mg/kg per day for 7 days [8, 9]. In this context, the development of neuro-protective compounds that can be administered together with colistin is important to increase its clinical use.

Flavonoids, a group of natural substances with different phenolic structures, are found in fruits, vegetables, roots, grains, bark, flowers, stems, wine and tea [10, 11]. The most common native flavonoid is rutin, which is vastly present in food such as apples, onions, red wine and tea [12]. Rutin has different protective effects in vitro as well as in vivo against oxidative stress-mediated diseases and lipid peroxidation [13, 14]. It has a wide range of pharmacological and biological effects including anti-inflammatory, antioxidant, antihypertensive, antiapoptotic, antiautophagic and neuroprotective activities in several experimental models of rodents [15–17].

Based on these knowledge, the present study was designed to investigate the neuro-protective effects of rutin against colistin-induced neurotoxicity in rats.

Materials and methods

Drug and chemicals

Colistin (Colimycin® 150 mg/flakon, Koçak Pharma, İstanbul, Turkey) was obtained from a local pharmacy. Rutin ($\geq 94\%$) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals used in this study were of analytical grade.

Animals

Male Sprague Dawley rats, weighing 220–250 g (8–10 weeks of age) were used in the study following the guidelines of the Local Animal Care Committee of Ataturk University, Erzurum, Turkey (Approval No: 2019-4/56). The rats were received from Ataturk University Experimental Research and Application Center. They were housed in plastic cages under controlled environmental conditions (humidity $45 \pm 5\%$, temperature 25 ± 1 °C and 12 h light/dark cycle) with free access to water and food.

Experimental protocol

The animals were separated into five different groups of seven rats each per plastic cages and were treated for 7 days as follows:

Group I (control): rats received normal saline (1 ml, p.o., once daily) for 7 days.

Group II (rutin): rats received rutin (100 mg/kg b.w., p.o.) for 7 days [18].

Group III (colistin): rats received colistin (15 mg/kg b. w., i.p., once daily) for 7 days [6].

Group IV (Col + Rut 50): rat received colistin (15 mg/kg b. w., i.p.) and rutin (50 mg/kg b. w., p.o.) for 7 days.

Group V (Col + Rut 100): rat received colistin (15 mg/kg b. w., i.p.) and rutin (100 mg/kg b. w., p.o.) for 7 days.

On day 8, rats were killed under mild sevoflurane anesthesia (Sevorane liquid 100%, Abbott Laboratory, İstanbul, Turkey), and brain tissues were removed immediately. Part of the brain was fixed in 10% formalin and embedded in paraffin for immunohistochemical and histopathological staining. The remained part of the brain was stored at -20 °C for biochemical and Real-Time PCR analyses.

Evaluation of biomarkers of oxidative stress

To obtain brain tissue homogenate, the tissue was grinded using liquid nitrogen. The tissues were homogenized in 1:10 (w/v) ratio of tissue and 1.15% potassium chloride buffer. A part of the homogenate was taken and centrifuged at 10,000 rpm for 20 min at 4 °C, and the obtained supernatant was used for the measurement of glutathione peroxidase (GPx) activity and glutathione (GSH) level. The remaining part was centrifuged at 3500 rpm for 15 min and supernatants were used for the assay of catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA). The CAT, GPx and SOD activities were assayed according to the methods prescribed by Aebi [19], Matkovics [20] and Sun et al. [21], respectively. The GPx and SOD activities were expressed as U/g protein. CAT activity has been described as katal/g protein. MDA levels were estimated according to Placer et al. [22]. GSH content was determined by the method of Sedlak and Lindsay [23]. The MDA and GSH levels have been expressed as nmol/g tissue. Protein amount in the brain was measured according to Lowry et al. [24].

Evaluation of AChE and BChE enzyme activities

The activities brain tissue Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were studied as described by Ellman et al. [25]. These enzyme activities were measured spectrophotometrically at a wavelength of 412 nm (UV-1800 Shimadzu, Kyoto, Japan) and the activities were expressed as U/mg protein.

Evaluation of inflammation markers

Neuronal nitric oxide synthase (nNOS), nuclear factor kappa B (NF-κB) and tumor necrosis factor-α (TNF-α) levels in brain tissue were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the

manufacturer's instructions (YL Biont, Shanghai, China). Brain homogenates required for all ELISA kits were obtained as described in our previous studies [18, 26].

Evaluation of apoptotic markers

p53 protein level and cysteine aspartate specific protease-3 (caspase-3) activity in the brain tissue was detected with a commercial rat ELISA kit (YL Biont) according to the manufacturer's instruction.

Evaluation of GFAP and Nrf2

Glial fibrillary acidic protein (GFAP) and nuclear factor erythroid 2-related factor 2 (Nrf2) levels were determined using a commercial rat ELISA kit purchased from YL Biont (Shangai, China) following the manufacturer's instruction.

Real time PCR assay

Total RNA was isolated from brain tissue using TRIZOL reagent (Invitrogen, Cat: 15,596,026, USA) following the manufacturer's instructions. cDNA synthesis was performed with QuantiTect Reverse Transcription (Qiagen, Cat:330,411 Germany) from total RNA samples according to the manufacturer's instructions [27]. Real-time quantitative PCR (RT-PCR) was performed to measure the mRNA transcript level of BDNF, Bcl-2, and Bax in the brain tissues using ROTOR-GENE Q 5plex HRM Real-Time PCR Detection System (Qiagen, Germany). GAPDH was used as internal control gene. The specificity of PCR amplification was confirmed by agarose gel electrophoresis and melting curve analysis [27, 28]. Relative folds of expressions were evaluated with the $2^{-\Delta\Delta CT}$ method [29]. All primer sequences and reaction conditions were shown in Table 1.

Histopathologic analysis

The rat brains were removed after necropsy and fixed in the 10% buffered formalin solutions for 3 days, and were then passed through alcohol-xylene processes and embedded into paraffin blocks. The 5-µm brain sections were taken into slides and rehydrated, and stained with hematoxylin at room temperature for 2 min and then with eosin for 10 s. Findings were evaluated in terms of necrotic and apoptotic cells under a light microscope (Olympus BX51-DP72) at ×20 magnification.

Immunohistochemistry staining

Following deparaffinization and hydration, brain sections were immersed to 3% H_2O_2 solution to inactivate endogenous peroxidase activity for 10 min. Then, the sections were boiled in sodium citrate buffer (pH 6.0) to unmask antigens for 10 min, and were cooled for 10 min. The sections were incubated with blocking solution at room temperature for 15 min to eliminate any nonspecific binding. The sections were incubated with cyclic AMP response element binding protein (CREB) Antibody (LB9) primary antibody (Novus Biologicals, Cat. No: NB100-74,393, Dilution: 1/200) and ERK 1/2 Antibody (C-9) primary antibody (Santa Cruze, Cat. No: sc-514302, Dilution: 1/250) for 30 min. Subsequently, the procedure of Expose mouse and rabbit specific HRP/DAB detection IHC kit (Abcam, No: ab80436) was followed. 3,3′-diaminobenzidine chromogen was used and counterstained with hematoxylin, dehydrated, and cover slipped. Positive cells were examined at ×20 magnification under a light microscope (Olympus BX-51, DP72). As negative controls. Sections incubated with PBS instead of the primary antibodies. ERK 1/2 and CREB immunopositivity were scored as follows: none (−), mild (+), moderate (++), and intense (+++).

Statistical analysis

The biochemical analysis was compared by one-way analysis of variance (ANOVA). Tukey's multiple comparison test were performed. $p < 0.05$ were considered statistically significant. The results are represented as mean ± SEM. For RT-PCR analysis, relative mRNA fold change graphics were created by using Graph pad prism software Inc., (Version 7.0, California, USA). Results are expressed as mean ± SEM.

Table 1 Primer sequences and RT-PCR conditions

Primer	Sequences (5′–3′)	Length (bp)	Accession no.	Reaction conditions
GAPDH	F: GTGCCAGCCTCGTCTCATA R: GATGGTGATGGGTTTCCCGT	248	NM_017008.4	94 °C for 15 s, 55 °C 30 s/72 °C 30 s. (40 cycle)
BDNF	F: CATAACCCCGCACACTCTGT R: TCACCTGGTGGAACTTCCCG	90	NM_001270638.1	94 °C for 15 s, 55 °C 30 s/72 °C 30 s. (40 cycle)
Bcl2	F: CTGGTGGACAACATCGCTCT R: GCATGCTGGGGCCATATAGT	155	NM_016993.1	94 °C for 15 s, 55 °C 30 s/72 °C 30 s. (40 cycle)
Bax	F: ACACCTGAGCTGACCTTGGA R: AGTTCATCGCCAATTCGCCT	115	NM_016993.1	94 °C for 15 s, 55 °C 30 s/72 °C 30 s. (40 cycle)

Statistically differences were considered to be significant at *p < 0.05, **p < 0.01 and ***p < 0.001. For immunohistochemical analysis, differences were analyzed with a nonparametric test (Kruskal–Wallis) followed by Mann–Whitney U test (p < 0.05). Data are presented as means (±) standard deviations (S.D.).

Results

Effect of rutin treatment on brain oxidative stress biomarkers

Treatment with colistin significantly reduced the activities of SOD, CAT and GPx enzyme, and level of GSH (p < 0.05) by 31%, 42%, 43%, and 41%, respectively as compared to the control group (Table 2). These biochemical parameters showed a significant increase in the Col + Rut 50 and Col + Rut 100 groups as compared with the colistin-treated group. But the higher dose of rutin (100 mg/kg b. wt.) significantly increased the activities of SOD, CAT, GPx, and GSH in Col + Rut 100 group as compared to colistin group.

Colistin administration significantly increased MDA level by 49%, comparison with control group. However, MDA levels decreased to 9% in Col + Rut 50 group and 22% in Col + Rut 100 group.

Effect of rutin treatment on brain cholinergic enzymes

In this study, we measured AChE and BChE activities to assess the protective effect of rutin against colistin-induced neurotoxicity. Brain AChE and BChE enzyme activities were found to increase by 65% and 70% in colistin-treated groups compared to control groups, respectively (p < 0.05). However, rutin treatment along with colistin significantly (p < 0.05) decreased the elevation of these enyme activities in dose dependent manner (Fig. 1a, b).

### Effect of rutin treatment on brain inflammation

Colistin induced a significant increase in NF-κB, TNF-α and nNOS levels as 0.42-, 0.7- and 0.47-folds respectively as compared to the control group (p < 0.05) (Fig. 2). Rutin treatment (50 and 100 mg/kg b.w.) along with colistin significantly decreased NF-κB levels by 0.10- and 0.20-fold, TNF-α levels by 0.15- and 0.29-fold and nNOS levels by

Table 2 The effects of rutin on biochemical parameters in colistin-induced neurotoxicity in rats

Parameters	Control	Rutin	Colistin	Col + Rut 50	Col + Rut 100
MDA (nmol/g tissue)	29.04 ± 0.52^a	27.90 ± 0.62^a	43.38 ± 0.77^d	39.55 ± 0.45^c	33.93 ± 0.68^b
SOD (U/g protein)	19.37 ± 0.45^d	21.04 ± 0.28^d	13.28 ± 0.36^a	15.52 ± 0.39^b	17.38 ± 0.61^c
CAT (katal/g protein)	12.89 ± 0.46^d	13.36 ± 0.54^d	7.48 ± 0.20^a	8.96 ± 0.10^b	10.61 ± 0.23^c
GPx (U/g protein)	17.79 ± 0.39^c	18.36 ± 0.46^c	10.28 ± 0.21^a	13.96 ± 0.43^b	15.02 ± 0.46^b
GSH (nmol/g tissue)	3.43 ± 0.05^c	3.51 ± 0.03^c	2.02 ± 0.03^a	2.89 ± 0.17^b	2.90 ± 0.07^b

All data were expressed as mean ± SEM. (a–d) Different superscript letters indicates statistical difference among the groups (p < 0.05)
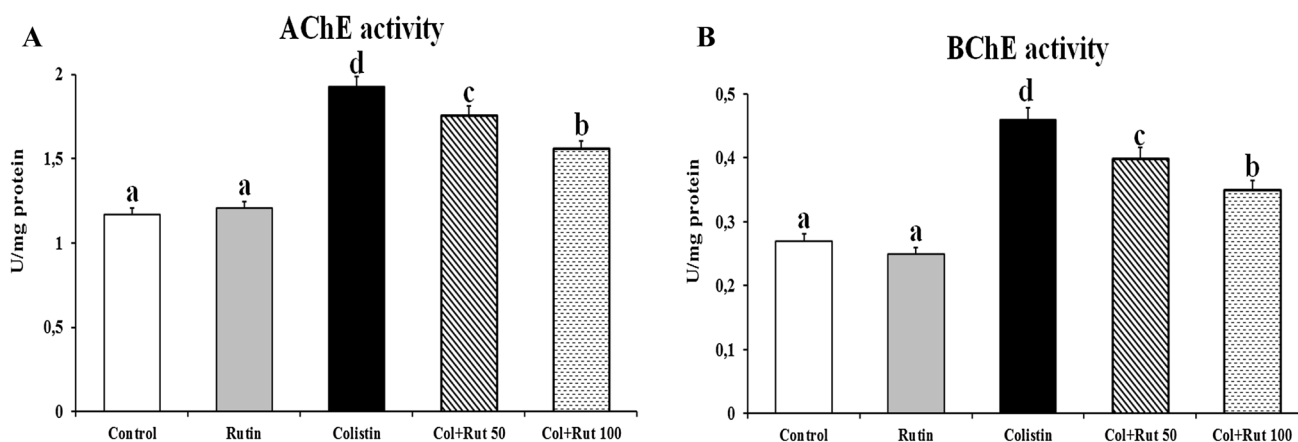
Fig. 1 **a** Effect of rutin on colistin-induced brain AChE activity in rats. **b** Effect of rutin on colistin-induced brain BChE activity in rats. All data were expressed as mean ± SEM. (a–d) Different letters indicate statistical difference among the groups (p < 0.05)
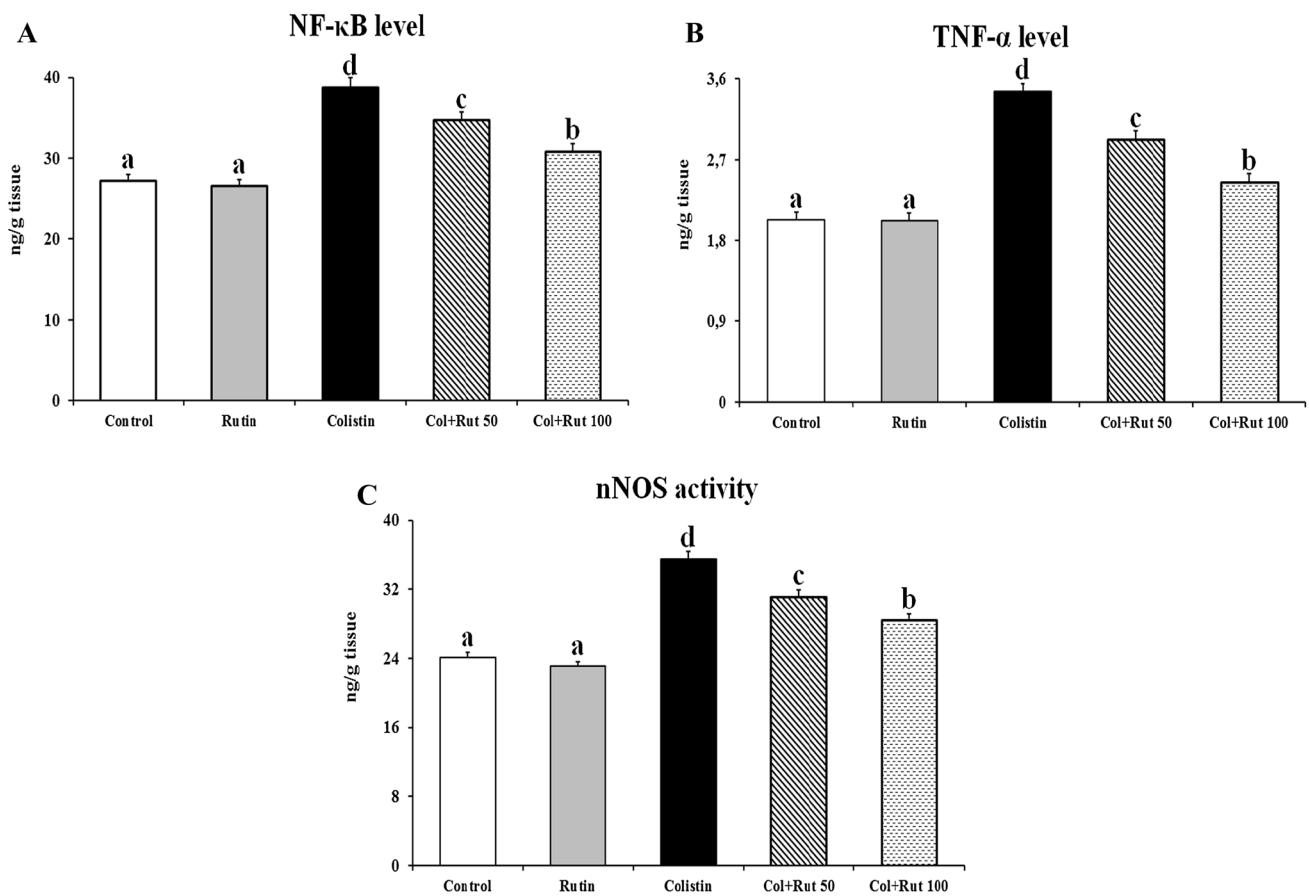
Fig. 2 a Effect of rutin on colistin-induced brain NF-κB level in rats. b Effect of rutin on colistin-induced brain TNF-α level in rats. c Effect of rutin on colistin-induced brain nNOS activity in rats. All data were expressed as mean ± SEM. (a–d) Different letters indicate statistical difference among the groups (p < 0.05)

0.12- and 0.20-fold as compared with the colistin-treated group.

Effect of rutin treatment on brain apoptosis

In the present study, the p53 level and caspase-3 activity in brain tissues were determined by ELISA. As indicated in Fig. 3, colistin significantly (p < 0.05) increased the level of p53 (+ 68%) and activity of caspase-3 (+ 70%) as compared to the control group. In contrast, rutin treatment significantly attenuated the colistin-increased level of p53 and activity of caspase-3 in a dose-dependent manner.

Effect of rutin treatment on GFAP and Nrf2 levels

The GFAP level was significantly increased (0.46-fold) in colistin-induced group compared to the control group (p < 0.05). Both doses of rutin (50 and 100 mg/kg b.w.) significantly decreased GFAP levels by 0.08- and 0.19-fold, compared to the colistin-treated group, respectively (Fig. 4a).

The Nrf2 level was significantly declined (0.29-fold) in colistin-treated group as compared to the control group (p < 0.05). The level of Nrf2 was considerably increased in Col + Rut 50 (0.13-fold) and Col + Rut 100 (0.21-fold) treated groups compared to the only colistin-treated rats (Fig. 4b).

Effect of rutin on the mRNA level of BDNF, Bax and Bcl-2

The expression levels of BDNF, Bax and Bcl-2 genes in the brains were determined by qRT-PCR. The expression level of BDNF was increased in the colistin group (p < 0.01), unchanged in rutin group (p > 0.05) in comparison to control group. BDNF expression levels were slightly up-regulated in the Col + Rutin 50 and Col + Rutin 100 groups compared to the control group (p > 0.05), but significantly down-regulated compared to the colistin group (p < 0.05) (Fig. 5a). Expression level of Bcl-2 decreased only in the colistin group (p < 0.01), almost remained unchanged (p > 0.05) in rutin group compared to the control group. Meanwhile,
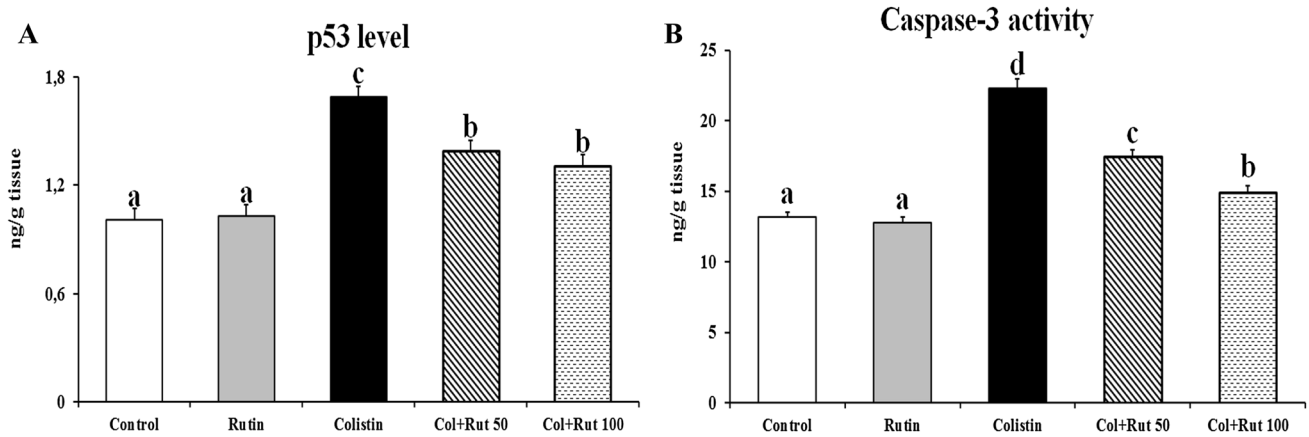
Fig. 3 **a** Effect of rutin on colistin-induced brain p53 level in rats. **b** Effect of rutin on colistin-induced brain caspase-3 activity in rats. All data were expressed as mean ± SEM. (a–d) Different letters indicate statistical difference among the groups (p < 0.05)
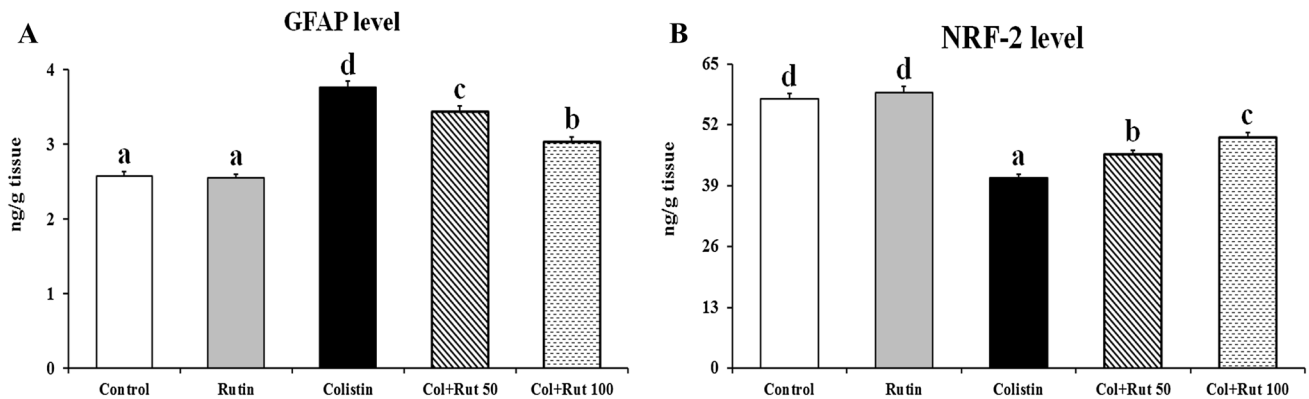
Fig. 4 **a** Effect of rutin on colistin-induced brain GFAP level in rats. **b** Effect of rutin on colistin-induced brain Nrf2 level in rats. All data were expressed as mean ± SEM. (a–d) Different letters indicate statistical difference among the groups (p < 0.05)

mRNA level of Bcl-2 was slightly down regulated in the Col + Rutin 50 and Col + Rutin 100 groups compared to the control group (p > 0.05), however it was significantly up-regulated compared to the colistin group (p < 0.05) (Fig. 5b). mRNA level of Bax was significantly up-regulated in the colistin group (p < 0.01), slightly increased in the colistin + rutin 50 and colistin + rutin 100 groups compared to the control group (p > 0.05), but it was down-regulated compared to the colistin (p < 0.05) (Fig. 5c).

Histopathology results

Figure 6a and b shows pyramidal cell layer, with normal neurons hippocampus of control and rutin-treated groups. Figure 6c shows necrotic pyramidal cell and apoptotic neurons in hippocampal area in colistin-treated group. Figure 6d shows moderate pyknotic changes and apoptotic neurons in hippocampal area of groups rutin-treated with colistin.

Figure 6e also shows that the necrotic and apoptotic neurons are considerably reduced in hippocampal area.

Effects of rutin on kinases 1 and 2 (ERK1/2) and CREB immunoreactivity

Results of ERK1/2 and CREB immunopositivity in the hippocampal neurons are shown in Fig. 7a–l. Figure 7f–l shows negative control in the hippocampal neurons. ERK1/2 and CREB immunoreactivity was determined in nucleus of neuronal cells of the brain tissues of control and treatment groups. We detected an intense immunopositivity for ERK1/2 activity in a substantial number of hippocampal neurons in control and rutin treated groups (Fig. 7a, b). This immunopositivity was significantly reduced in the colistin group (Fig. 7c). It was observed that colistin combined with different doses of rutin (50 and 100 mg/kg) increased ERK 1/2 immunopositivity in hippocampal neurons (Fig. 7d, e). Also, the number of CREB-immunopositive neuronal cells
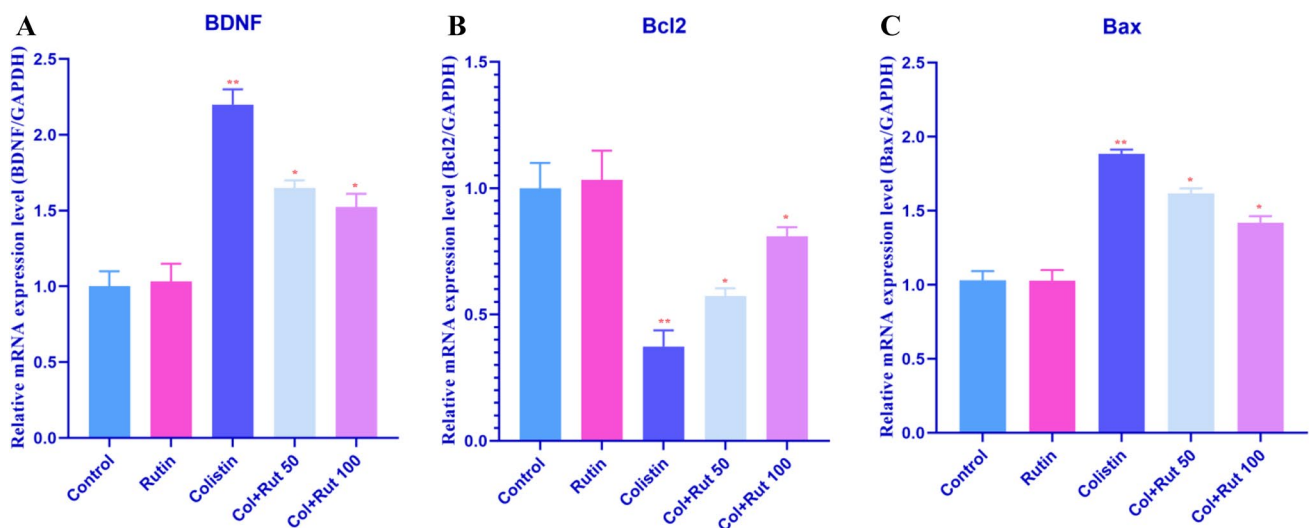
Fig. 5 The mRNA transcript level of BDNF, Bcl-2, and Bax in the brain of rats. **a** Represent the relative mRNA expression levels of BDNF. **b** Represent the relative mRNA expression levels of Bcl-2.C) Represent the relative mRNA expression levels of Bax. Values represent the mean $\pm$ SD of 3 independent samples; error bars indicate standard deviation. Statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001) was analyzed using One Way ANOVA
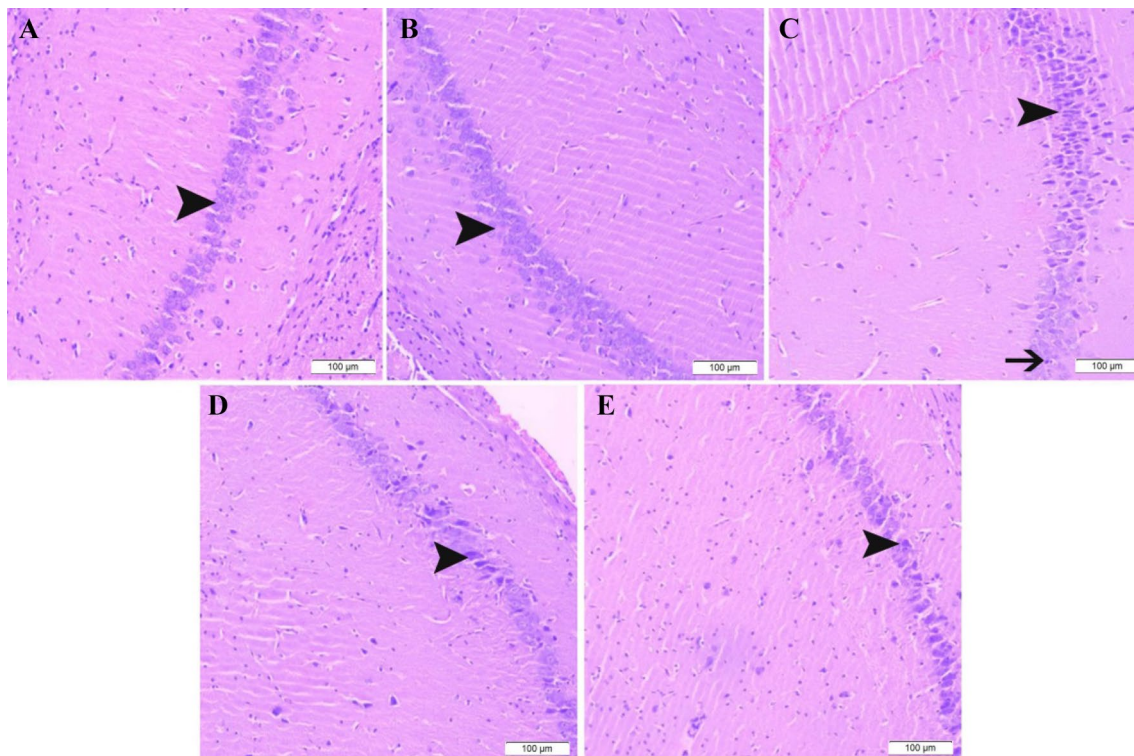
Fig. 6 Effects of rutin on the number of surviving neurons in the hippocampal region following colistin administration in rats. **a, b** Photomicrograph of hippocampus of control and rutin-treated rats showing normal pyramidal cell layer, with normal neurons (arrowhead). **c** Photomicrograph of hippocampus of colistin-treated rat showing intense necrotic of pyramidal cell layer (arrowhead), with increased apoptotic neurons (arrow). **d, e** Photomicrograph of the hippocampus of a colistin-treated rat receiving rutin 50 and rutin 100 doses showing reduction in the number of necrotic cells (arrowhead) in the pyramidal cell layer. H&E; scale bar, 100 µm
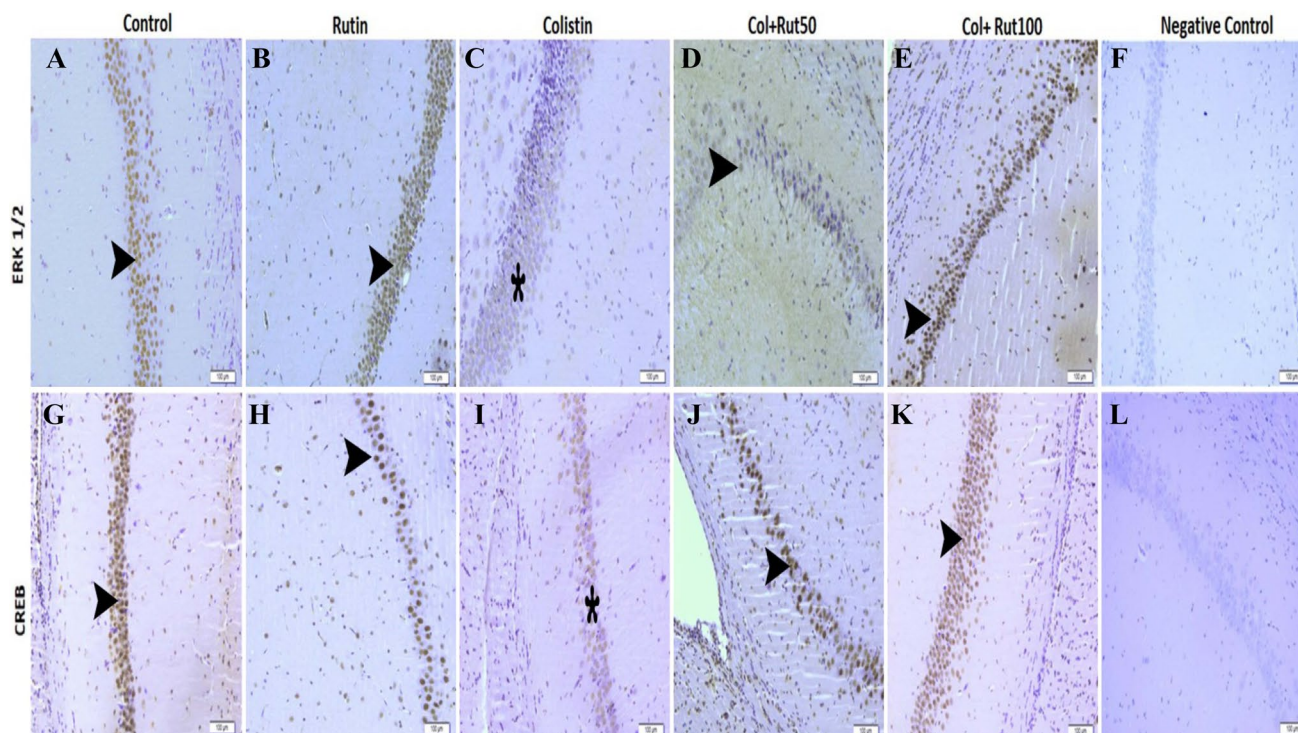
Fig. 7 Rutin which was applied together with colistin upregulated the decreasing levels of CREB and ERK1/2 in the colistin group. Negative controls (**f**, **l**). Immunohistochemical study using anti-ERK 1/2 and anti-CREB antibodies showed a intense immunoreactivity in hippocampal neurons (arrowhead) of control and rutin group rats (**a**, **b**, **g**, **h**). A noticeably mild immunoreactivity of ERK 1/2 and CREB was found in hippocampal neurons (arrowhead) of colistin group rats (**c**, **i**). Low dose (50 mg/kg) and high dose rutin (100 mg/kg) increased the immunoreactivity of ERK1/2 and CREB in hippocampal neurons (arrowhead) of Col + Rut 50 and Col + Rut 100 group rats (**d**, **e**, **j**, **k**). IHC; scale bar, 100 µm

in the hippocampus in the control and rutin treated groups was significantly intense compared with that in the colistin group (Fig. 7g–i). It was observed that rutin administration (50 and 100 mg/kg) with colistin increased CREB reactivity in hippocampal neurons (Fig. 7j, k).

In terms of ERK1/2 and CREB immunopositivity, no statistically remarkable difference was identified in the brains of the rats in the control, rutin and Col + Rutin 100 groups. Although there was no considerable difference between colistin and Col + Rutin 50 groups in ERK 1/2 immunopositivity, there was a significant difference between colistin and other groups in CREB immunopositivity (p < 0.05, Table 3).

Discussion

Neurotoxicity is an important undesirable effect of polymyxin therapy. Therefore, the discovery of effective neuroprotective natural compounds for co-administration during colistin treatment is critical to prolong the clinical usage of this important cationic polypeptide antibiotic. In present study, we demonstrated that rutin exhibits neuro-protective

Table 3 Expressions of ERK 1/2 and CREB in rat brains that are exposed to colistin and rutin

Groups	ERK 1/2	CREB
Control	2.57 ± 0.78^a	2.85 ± 0.37^a
Rutin	2.85 ± 0.37^a	2.71 ± 0.48^a
Colistin	1.00 ± 0.81^b	0.85 ± 0.69^b
Col + Rut 50	1.57 ± 0.53^b	2.42 ± 0.53^a
Col + Rut 100	2.42 ± 0.78^a	2.57 ± 0.53^a

All data were expressed as mean ± SD. Different superscript letters (a and b) indicates statistical difference among the groups (p < 0.05)

properties by mitigating oxidative stress, apoptosis and neuroinflammation in colistin-induced neurotoxicity.

The nervous system is delicate to oxidative stress owing to its high polyunsaturated fatty acid content and high oxygen demand [30]. Oxidative stress has a major role in the pathogenesis of neurodegenerative disorders [6]. Increased generation of reactive oxygen species (ROS) is a significant mechanism in colistin-induced neurotoxicity [6, 7, 9]. Notably, excessive ROS can result in the protein denaturation, peroxidation in membrane lipids, damage in nucleic acids,

and they could alter neuronal function and impair nervous system [31–33]. Recent studies have showed ROS-mediated oxidative stress plays a major role in colistin-induced in vitro neurotoxicity [34, 35]. Nrf2 as a known transcription factor and the key regulator of the cellular response of oxidative stress has been reported to stimulate the levels of antioxidant enzymes [36]. The activation of Nrf2 reduces cellular oxidative damage by activating genes that encode antioxidant enzymes, such as heme oxygenase-1 (HO-1), CAT, SOD and GPx [37]. In our study, colistin significantly reduced SOD, CAT, GPx, GSH and Nrf2 levels in brain tissue and increased MDA content (Table 2 and Fig. 4b). Rutin works as a scavenger of ROS by donating hydrogen atoms to superoxide anions, peroxy radicals and hydroxyl radicals [18]. In present study, rutin efficiently reduced MDA level, increasing CAT, GPx, SOD, GSH and Nrf2 levels in colistin-induced neurotoxicity. Our in vivo findings were in agreement with previous studies that revealed the antioxidant properties of the rutin [17, 38].

AChE is a serine protease enzyme that hydrolyzes the neurotransmitter acetylcholine. It is mainly found in neuromuscular junctions and plays an important role in the central nervous system of cholinergic brain synapses [39, 40]. On the other hand, BChE is a serine hydrolase enzyme that hydrolyzes choline and non-choline esters and is expressed in all brain regions such as cerebellum, hippocampus and temporal cortex [41, 42]. Cholinergic neurons are essential for memory function and learning in the cerebral cortex and thus AChE and BChE play a key role in this event [6, 43]. In a previous study, it was reported that colistin increased AChE and BChE enzyme activities [6]. In present study, treatment of rutin decreased AChE and BChE enzyme activities compared with the colistin-treated group. OH groups of flavonoids have been reported to interact with the peripheral anionic region of AChE and prevents entry to the active site of the enzyme. In a study, accessible binding region of rutin was shown in AChE. The rutin-AChE interaction was stabilised through hydrogen bonds, van der Waals and hydrophobic interactions resulting a dose-dependent inhibition of the enzyme [44, 45].

Increasing evidence has showed that ROS activates pro-inflammatory mediators, such as NF-κB and TNF-α and subsequently induces neuroinflammation in brain [46]. TNF-α is a pro-inflammatory cytokine and a key mediator of inflammatory tissue damage [7]. Under pathological conditions, microglia release high levels of TNF-α, and overproduction of TNF-α is a significant component of the neuroinflammatory response related to some neurological disorders [47]. Moreover, NF-κB is a redox transcription factor, which serves as a crucial regulator of inducible expression of genes involved in inflammation and various autoimmune disease [48]. Expression of TNF-α, inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β) and cyclooxygenase-2

(COX-2) can be altered by the activation of NF-κB [48–50]. Thus, inhibition of NF-κB may provide a potential approach for the reduction of neuroinflammation. Nitric oxide (NO) is an important molecule that mediates the neurotoxicity, neurotransmission, and vasodilation [51]. However, excessive NO generation by the nNOS enzyme under neuroinflammation conditions result in the neuronal cell death and production of reactive nitrogen species [52]. In the present study, we found that rutin considerably inhibited the levels of NF-κB, TNF-α and nNOS in colistin-induced neurotoxicity. Recently, a study found that rutin reduced the TNF-α, IL-1β and IL-6 levels in sodium fluoride (NaF) induced neurotoxicity in rats [53].

It is well known that mitochondria play an important role in cellular redox homeostasis and induction of apoptotic process [54]. Colistin induced a rapid dissipation of the mitochondrial membrane potential, indicative of mitochondrial dysfunction. Pro- and anti-apoptotic Bcl-2 family proteins including Bax and Bcl-2 regulate the mitochondrial pathway [34]. Colistin increases mitochondrial outer membrane permeability by causing an increase in Bax/Bcl-2 ratios and cytochrome c release. Release of cytochrome c into the cytoplasm causes activation of casapase-3 and -9, which triggers apoptosis [34, 35]. In our study, colistin significantly increased p53 protein levels. In this context, p53 has been reported to activate Bax penetrating the mitochondria, causing mitochondrial cytochrome c release and activation of caspase enzymes, which induces apoptosis [55]. In present study, we observed the down-regulation of Bcl-2 expression as well as up-regulation of p53, caspase-3 and Bax expressions in colistin-induced brain tissues. It has been reported in a previous study that rutin ameliorates brain tissues against ethanol-induced apoptosis and causes an decrease in caspase-3, Bax, and cytochrome c expressions [56].

The ERK1/2 are members of the mitogen-activated protein kinase (MAPK) family. ERK1/2 pathway can be activated in response to oxidative stress, growth factors, cytokines, chemokines and ischemic injury [57]. Inhibition of the ERK1/2 pathway has been reported to reduce brain injury and focal infarction volume in mice after ischemia [58]. On the other hand, GFAP is known as an important component of the astrocyte cytoskeleton during brain development [59]. This component is necessary for astrocyte functioning, particularly in formation of blood–brain barrier and in regulation of central nervous system activities of the developing brain [60]. However, increased GFAP expression causes brain injury and inflammatory neurological disorders [59]. Our results showed that exposure to colistin remarkably reduced ERK1/2 expression while increased GFAP levels in brain tissue. However, rutin treatment significantly suppressed the GFAP level while increased ERK1/2 expression.

CREB is a transcription activator involved in physiological signals such as depolarization, neurotransmitters, factors

controlling differentiation, mitogenic signals, synaptic activity, and stressors [61]. BDNF is an important neurotrophic factor that participate in various intracellular signaling processes, neuronal protection and survival, dendritic and axonal morphology and synaptic plasticity [62]. Transcription of BDNF is induced by activation of CREB [61]. In this study, considerable increase in the mRNA levels of BDNF and CREB expression were observed in colistin-treated rat brain tissues. However, rutin given for treatment significantly decreased expressions of CREB and BDNF. It is has been reported that rutin may protect hippocampus against beta-amyloid-induced damage via up regulation of CREB and BDNF [63].

Conclusion

In conclusion, we demonstrated that rutin supplementation alleviated colistin-induced neurotoxicity in male rats. The probable mechanisms underlying these protective effects have been demonstrated to be associated with the antioxidant, anti-apoptotic and anti-inflammatory effects of rutin in brain tissues. In addition, this study is thought to pave the way for further studies to examine other machineries underlying the neuroprotective effects of rutin.

Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

References

1. Wang J, Yi M, Chen X, Muhammad I, Liu F, Li R, Li J, Li J (2016) Effects of colistin on amino acid neurotransmitters and blood-brain barrier in the mouse brain. Neurotoxicol Teratol 55:32–37
2. Falagas ME, Kasiakou SK, Saravolatz LD (2005) Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 40(9):1333–1341
3. Aksu EH, Kandemir FM, Küçükler S, Mahamadu A (2018) Improvement in colistin-induced reproductive damage, apoptosis, and autophagy in testes via reducing oxidative stress by chrysin. J Biochem Mol Toxicol 32(11):e22201
4. Liu Y, Dai C, Gao R, Li J (2013) Ascorbic acid protects against colistin sulfate-induced neurotoxicity in PC12 cells. Toxicol Mech Methods 23(8):584–590
5. Hanedan B, Ozkaraca M, Kirbas A, Kandemir FM, Aktas MS, Kilic K, Comakli S, Kucukler S, Bilgili A (2018) Investigation of the effects of hesperidin and chrysin on renal injury induced by colistin in rats. Biomed Pharmacother 108:1607–1616
6. Ajiboye T (2018) Colistin sulphate induced neurotoxicity: studies on cholinergic, monoaminergic, purinergic and oxidative stress biomarkers. Biomed Pharmacother 103:1701–1707
7. Edrees NE, Galal AA, Monaem ARA, Beheiry RR, Metwally MM (2018) Curcumin alleviates colistin-induced nephrotoxicity and neurotoxicity in rats via attenuation of oxidative stress, inflammation and apoptosis. Chem-Biol Interact 294:56–64
8. Dai C, Li J, Lin W, Li G, Sun M, Wang F, Li J (2012) Electrophysiology and ultrastructural changes in mouse sciatic nerve associated with colistin sulfate exposure. Toxicol Mech Methods 22(8):592–596
9. Dai C, Li J, Li J (2013) New insight in colistin induced neurotoxicity with the mitochondrial dysfunction in mice central nervous tissues. Exp Toxicol Pathol 65(6):941–948
10. Panche A, Diwan A, Chandra S (2016) Flavonoids: an overview. J Nutr Sci. https://doi.org/10.1017/jns.2016.41
11. Kuzu M, Kandemir FM, Yildirim S, Kucukler S, Caglayan C, Turk E (2018) Morin attenuates doxorubicin-induced heart and brain damage by reducing oxidative stress, inflammation and apoptosis. Biomed Pharmacother 106:443–453
12. Ola MS, Ahmed MM, Ahmad R, Abuohashish HM, Al-Rejaie SS, Alhomida AS (2015) Neuroprotective effects of rutin in streptozotocin-induced diabetic rat retina. J Mol Neurosci 56(2):440–448
13. Alhoshani AR, Hafez MM, Husain S, Al-sheikh AM, Alotaibi MR, Al Rejaie SS, Alshammari MA, Almutairi MM, Al-Shabanah OA (2017) Protective effect of rutin supplementation against cisplatin-induced nephrotoxicity in rats. BMC Nephrol 18(1):194
14. Aksu E, Kandemir F, Özkaraca M, Ömür A, Küçükler S, Çomaklı S (2017) Rutin ameliorates cisplatin-induced reproductive damage via suppression of oxidative stress and apoptosis in adult male rats. Andrologia 49(1):e12593
15. Almutairi MM, Alanazi WA, Alshammari MA, Alotaibi MR, Alhoshani AR, Al-Rejaie SS, Hafez MM, Al-Shabanah OA (2017) Neuro-protective effect of rutin against Cisplatin-induced neurotoxic rat model. BMC Complement Altern Med 17(1):472
16. Caglayan C, Kandemir FM, Darendelioğlu E, Yıldırım S, Kucukler S, Dortbudak MB (2019) Rutin ameliorates mercuric chloride-induced hepatotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. J Trace Elem Med Biol 56:60–68
17. Kandemir FM, Ozkaraca M, Yildirim BA, Hanedan B, Kirbas A, Kilic K, Aktas E, Benzer F (2015) Rutin attenuates gentamicin-induced renal damage by reducing oxidative stress, inflammation, apoptosis, and autophagy in rats. Ren Fail 37(3):518–525
18. Caglayan C, Kandemir FM, Yildirim S, Kucukler S, Eser G (2019) Rutin protects mercuric chloride-induced nephrotoxicity via targeting of aquaporin 1 level, oxidative stress, apoptosis and inflammation in rats. J Trace Elem Med Biol 54:69–78
19. Aebi H (1984) [13] Catalase in vitro. In: Packer L (ed) Methods in enzymology, vol 105, Elsevier, Amsterdam, pp 121–126
20. Matkovics B (1988) Determination of enzyme activity in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika 15:248–250
21. Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34(3):497–500
22. Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem 16(2):359–364
23. Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25:192–205
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
25. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88–95

26. Kandemir FM, Yildirim S, Caglayan C, Kucukler S, Eser G (2019) Protective effects of zingerone on cisplatin-induced nephrotoxicity in female rats. Environ Sci Pollut Res 26:22562–22574

27. Özdemir S, Çomaklı S (2018) Investigation of the interaction between bta-miR-222 and the estrogen receptor alpha gene in the bovine ovarium. Reprod Biol 18(3):259–266

28. Arslan H, Altun S, Özdemir S (2017) Acute toxication of deltamethrin results in activation of iNOS, 8-OHdG and up-regulation of caspase 3, iNOS gene expression in common carp (*Cyprinus carpio* L.). Aquat Toxicol 187:90–99

29. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4):402–408

30. Dai C, Ciccotosto GD, Cappai R, Tang S, Li D, Xie S, Xiao X, Velkov T (2018) Curcumin attenuates colistin-induced neurotoxicity in N2a cells via anti-inflammatory activity, suppression of oxidative stress, and apoptosis. Mol Neurobiol 55(1):421–434

31. Benzer F, Kandemir FM, Kucukler S, Comaklı S, Caglayan C (2018) 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 124(5):448–457

32. Dai C, Tang S, Deng S, Zhang S, Zhou Y, Velkov T, Li J, Xiao X (2015) Lycopene attenuates colistin-induced nephrotoxicity in mice via activation of the Nrf2/HO-1 pathway. Antimicrob Agents Chemother 59(1):579–585

33. Jiang G-Z, Li J-C (2014) Protective effects of ginsenoside Rg1 against colistin sulfate-induced neurotoxicity in PC12 cells. Cell Mol Neurobiol 34(2):167–172

34. Dai C, Tang S, Velkov T, Xiao X (2016) Colistin-induced apoptosis of neuroblastoma-2a cells involves the generation of reactive oxygen species, mitochondrial dysfunction, and autophagy. Mol Neurobiol 53(7):4685–4700

35. Dai C, Ciccotosto GD, Cappai R, Wang Y, Tang S, Hoyer D, Schneider EK, Velkov T, Xiao X (2017) Rapamycin confers neuroprotection against colistin-induced oxidative stress, mitochondria dysfunction, and apoptosis through the activation of autophagy and mTOR/Akt/CREB signaling pathways. ACS Chem Neurosci 9(4):824–837

36. Zeng J, Chen Y, Ding R, Feng L, Fu Z, Yang S, Deng X, Xie Z, Zheng S (2017) Isoliquiritigenin alleviates early brain injury after experimental intracerebral hemorrhage via suppressing ROS-and/or NF-κB-mediated NLRP3 inflammasome activation by promoting Nrf2 antioxidant pathway. J Neuroinflammation 14(1):119

37. Dai C, Li B, Zhou Y, Li D, Zhang S, Li H, Xiao X, Tang S (2016) Curcumin attenuates quinocetone induced apoptosis and inflammation via the opposite modulation of Nrf2/HO-1 and NF-kB pathway in human hepatocyte L02 cells. Food Chem Toxicol 95:52–63

38. Magalingam KB, Radhakrishnan A, Haleagrahara N (2013) Rutin, a bioflavonoid antioxidant protects rat pheochromocytoma (PC-12) cells against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. Int J Mol Med 32(1):235–240

39. Akinyemi AJ, Oboh G, Fadaka AO, Olatunji BP, Akomolafe S (2017) Curcumin administration suppress acetylcholinesterase gene expression in cadmium treated rats. Neurotoxicology 62:75–79

40. Bayindir S, Caglayan C, Karaman M, Gülcin İ (2019) The green synthesis and molecular docking of novel N-substituted rhodanines as effective inhibitors for carbonic anhydrase and acetylcholinesterase enzymes. Bioorg Chem 90:103096

41. Maurice T, Strehaiano M, Siméon N, Bertrand C, Chatonnet A (2016) Learning performances and vulnerability to amyloid toxicity in the butyrylcholinesterase knockout mouse. Behav Brain Res 296:351–360

42. Taslimi P, Kandemir FM, Demir Y, İleritürk M, Temel Y, Caglayan C, Gulçin İ (2019) The antidiabetic and anticholinergic effects of chrysin on cyclophosphamide-induced multiple organ toxicity in rats: Pharmacological evaluation of some metabolic enzyme activities. J Biochem Mol Toxicol 33:e22313

43. Caglayan C (2019) The effects of naringin on different cyclophosphamide-induced organ toxicities in rats: investigation of changes in some metabolic enzyme activities. Environ Sci Pollut Res 26:26664–26673

44. Anesti M, Stavropoulou N, Atsopardi K, Lamari FN, Panagopoulos NT, Margarity M (2020) Effect of rutin on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol-treated mice. Epilepsy Behav 102:106632

45. Yan X, Chen T, Zhang L, Du H (2018) Study of the interactions of forsythiaside and rutin with acetylcholinesterase (AChE). Int J Biol Macromol 119:1344–1352

46. Wu Y-Q, Dang R-L, Tang M-M, Cai H-L, Li H-D, Liao D-H, He X, Cao L-J, Xue Y, Jiang P (2016) Long chain omega-3 polyunsaturated fatty acid supplementation alleviates doxorubicin-induced depressive-like behaviors and neurotoxicity in rats: involvement of oxidative stress and neuroinflammation. Nutrients 8(4):243

47. Olmos G, Lladó J (2014) Tumor necrosis factor alpha: a link between neuroinflammation and excitotoxicity. Mediators Inflamm 2014:861231

48. Kim YE, Hwang CJ, Lee HP, Kim CS, Son DJ, Ham YW, Hellström M, Han S-B, Kim HS, Park EK (2017) Inhibitory effect of punicalagin on lipopolysaccharide-induced neuroinflammation, oxidative stress and memory impairment via inhibition of nuclear factor-kappaB. Neuropharmacology 117:21–32

49. Caglayan C, Kandemir FM, Yıldırım S, Kucukler S, Kılınc MA, Saglam YS (2018) 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 102:517–530

50. Kandemir FM, Yildirim S, Kucukler S, Caglayan C, Mahamadu A, Dortbudak MB (2018) Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney. Biomed Pharmacother 105:981–991

51. Hu Z, Wang W, Ling J, Jiang C (2016) α-Mangostin inhibits α-synuclein-induced microglial neuroinflammation and neurotoxicity. Cell Mol Neurobiol 36(5):811–820

52. Çelik H, Kucukler S, Çomaklı S, Özdemir S, Caglayan C, Yardım A, Kandemir FM (2020) Morin attenuates ifosfamide-induced neurotoxicity in rats via suppression of oxidative stress, neuroinflammation and neuronal apoptosis. Neurotoxicology 76:126–137

53. Nkpaa KW, Onyeso GI (2018) Rutin attenuates neurobehavioral deficits, oxidative stress, neuro-inflammation and apoptosis in fluoride treated rats. Neurosci Lett 682:92–99

54. Cheng G, Kong Rh, Lm Z, Jn Z (2012) Mitochondria in traumatic brain injury and mitochondrial-targeted multipotential therapeutic strategies. Br J Pharmacol 167(4):699–719

55. Wu H-J, Pu J-L, Krafft PR, Zhang J-M, Chen S (2015) The molecular mechanisms between autophagy and apoptosis: potential role in central nervous system disorders. Cell Mol Neurobiol 35(1):85–99

56. Song K, Kim S, Na J-Y, Park J-H, Kim J-K, Kim J-H, Kwon J (2014) Rutin attenuates ethanol-induced neurotoxicity in hippocampal neuronal cells by increasing aldehyde dehydrogenase 2. Food Chem Toxicol 72:228–233

57. Cheng P, Alberts I, Li X (2013) The role of ERK1/2 in the regulation of proliferation and differentiation of astrocytes in developing brain. Int J Dev Neurosci 31(8):783–789

58. Zhang F, Wu Y, Jia J, Hu Y-S (2010) Pre-ischemic treadmill training induces tolerance to brain ischemia: involvement of glutamate and ERK1/2. Molecules 15(8):5246–5257

59. Tripathi S, Kushwaha R, Mishra J, Gupta MK, Kumar H, Sanyal S, Singh D, Sanyal S, Sahasrabuddhe AA, Kamthan M (2017) Docosahexaenoic acid up-regulates both PI 3K/AKT-dependent FABP 7–PPAR γ interaction and MKP 3 that enhance GFAP in developing rat brain astrocytes. J Neurochem 140(1):96–113

60. Hol EM, Pekny M (2015) Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Curr Opin Cell Biol 32:121–130

61. Hu Y, Liu M-Y, Liu P, Dong X, Boran AD (2014) Neuroprotective effects of 3, 6′-disinapoyl sucrose through increased BDNF levels and CREB phosphorylation via the CaMKII and ERK1/2 pathway. J Mol Neurosci 53(4):600–607

62. Pláteník J, Fišar Z, Buchal R, Jirák R, Kitzlerová E, Zvěřová M, Raboch J (2014) GSK3β, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. Prog Neuro-Psychopharmacol Biol Psychiatry 50:83–93

63. Moghbelinejad S, Nassiri-Asl M, Farivar TN, Abbasi E, Sheikhi M, Taghiloo M, Farsad F, Samimi A, Hajiali F (2014) Rutin activates the MAPK pathway and BDNF gene expression on beta-amyloid induced neurotoxicity in rats. Toxicol Lett 224(1):108–113