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Thymoquinone and geraniol alleviate cisplatin-induced neurotoxicity in rats through downregulating the p38 MAPK/STAT-1 pathway and oxidative stress

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ABSTRACT

Aims: Cisplatin (CP) is a widely used broad-spectrum antineoplastic agent used to treat a variety of human malignancies. Neurotoxicity is clinically evident in patients who have undergone a full course of chemotherapy. The aim of this study was to investigate the possible protective effects of thymoquinone (TQ) and geraniol (Ger) against CP-induced neurotoxicity in rats.
Main methods: Forty male Wistar albino rats were allocated into four groups as follows: normal control, CP-induced neurotoxicity, CP + TQ and CP + Ger.
Key findings: Our results demonstrated that simultaneous treatment with either TQ or Ger and CP significantly abrogated oxidative stress and downregulated the apoptotic markers p38 mitogen-activated protein kinase (MAPK), STAT-1, p53, p21 and MMP9; FMO3, however, was insignificantly decreased. In addition to the biochemical results, we assessed the histopathological findings, which confirmed the protective effect of TQ and Ger against the brain damage induced by CP.
Significance: The results of the present study indicate that simultaneous treatment with either TQ or Ger as natural antioxidants can provide protection against cisplatin-induced neurotoxicity in rats by attenuating oxidative stress and cell apoptosis.

1. Introduction

Cisplatin (CP) (cis-diamine-dichloro-platinum 11) is a broad-spectrum antineoplastic agent widely used for treating a variety of human malignancies, including carcinomas of the lung, colorectal, breast, head/neck, ovary, testis and bladder [1]. Despite being highly effective, many side effects, including nephrotoxicity, bone marrow toxicity, neurotoxicity, ototoxicity and germ cell toxicity, are associated with CP [2]. Approximately 30% of patients treated with CP have neurotoxicity because it can cross the blood-brain barrier and accumulate with repeated dosing [3–5]. The cytotoxic effect of CP results from the formation of a DNA adduct that causes cell death [6]. Although DNA is accepted as the major target of CP, only approximately 5–10% of the intracellular concentration of CP is found in the DNA fraction, while 75–85% of CP interacts with cytoplasmic targets, such as thiol peptides, proteins and RNA [7]. CP exposure further enhances the formation of reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which interact with DNA, lipids and proteins, causing DNA damage and lipid peroxidation [8].

CP administration activates mitogen-activated protein kinase (MAPK) pathways through ROS production and cross-linking with DNA, thus leading to apoptotic cell death [9]. MAPKs are a family of proline-serine threonine kinases that are activated in response to external stimuli that induce gene expression [10]. Several studies have demonstrated that the activation of the MAPK family by high doses of CP leads to the activation of other pathways, such as the JNK/SAPK, ERK and p38 MAPK pathways, which further phosphorylate the tumour suppressor gene p53, resulting in its activation and stabilization. In response to a great variety of stimuli, the MAPK pathway acts as a proapoptotic factor [11,12].

Many studies have explored the antioxidant effects of some natural plants that can effectively protect against the oxidative stress milieu induced by some drugs. One of the most powerful natural antioxidants that has been tested in many studies is thymoquinone (TQ; 2-isopropyl-

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5-methyl-1,4-benzoquinone). TQ is the principal active ingredient of the volatile oil derived from *Nigella sativa* (black cumin or black seed) [13]. TQ has been previously found to have many therapeutic activities, such as anti-inflammatory, antioxidant, anti-cancer, anti-bacterial, hypoglycaemic, anti-ulcerogenic, analgesic and neuroprotective effects [14]. TQ is also known to possess strong antioxidant properties through its scavenging activity against ROS, as it neutralizes superoxide anions and increases the levels of natural antioxidants [15]. Another natural antioxidant that has proven activity against oxidative stress is geraniol (Ger) (3,7-dimethyl-2,6-octadien-1-ol). Ger is an acyclic monoterpene alcohol that is the main constituent of the essential oils of rose, palmarosa, lavender, ginger, lemon and orange [16]. Ger has been reported to have multiple biological and pharmacological effects, such as anti-microbial, anti-inflammatory, antioxidant, anti-ulcer and neuroprotective properties [17,18].

We hypothesized that treatment with either TQ or Ger may abolish the neurotoxic effect induced by CP via their antioxidant effect and that these compounds may downregulate apoptotic markers in brain tissues.

Thus, the aim of the present study was to investigate the antioxidant and neuroprotective effects of TQ and Ger on CP-induced neurotoxicity in a rat model by evaluating the antioxidant and lipid peroxidation status and apoptotic marker gene expression in the brain tissues of affected rats.

2. Materials and methods

2.1. Drugs and chemicals

Cisplatin was purchased from Mylan S.A.S. (Saint-Priest, France), and TQ and Ger were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Corn oil was purchased from a local market.

2.2. Animals

Forty male albino Wistar rats aged 2–3 months, weighing 150–200 g were obtained from the animal facility of El Nahda University (Beni-Suef, Egypt). The animals were housed in polyacrylic cages in standard laboratory conditions (24 ± 1 °C; relative humidity, approximately 75 ± 5%; light-dark cycle, 12:12 h) with free access to food and tap water for one week before the experiment. The protocol of the current study was approved by the Experimental Animal Ethics Committee of Beni-Suef University (BSU/FS/2018/13) and all procedures for agent administration, blood, and tissue collection were in accordance with the National Institutes of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

2.3. Experimental design

Rats were divided randomly into four groups (ten rats in each group) and treated as follows: Group I (normal control group): rats were given corn oil at a dose of 2 ml/kg/day orally for 33 consecutive days [19] and injected intraperitoneally (i.p.) with saline at a dose of 2 ml/kg twice a week (a total of nine injections). Group II: rats were injected i.p. with 2 mg/kg body weight CP twice a week starting on the 5th day for 33 days (a total of nine injections) [20]. Group III: rats received TQ orally at a dose of 20 mg/kg body weight in corn oil three times a week [21], and the rats were injected i.p. with 2 mg/kg body weight CP twice a week starting on the 5th day for 33 days (a total of nine injections). Group IV: rats were treated orally with Ger at a dose of 100 mg/kg body weight in corn oil for 33 consecutive days [22], and the rats were injected i.p. with 2 mg/kg body weight CP twice a week starting on the 5th day for 33 days (a total of nine injections).

Twenty-four hours after the last injection of CP, the rats were fasted overnight and then sacrificed by decapitation. The brain tissues were collected, weighed, washed with phosphate-buffered saline and divided into three parts. The first part was used for Western blotting and real-time polymerase chain reaction (RT-PCR) analyses. The second part was used for histopathological examination and was stored in 10% formalin. The third part was stored at −80 °C for biochemical measurements of oxidative stress and antioxidant parameters.

2.4. Methods

2.4.1. Western blot analysis

Brain samples were prepared as described before; 50 mg of protein was extracted and homogenized in RIPA lysis buffer (50 mmol/l Tris-HCl, pH adjusted to 8.0, 150 mmol/l NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS)). To the homogenates, 2× Laemmli sample buffer was added and centrifuged for 30 min at 4 °C. Then, the supernatants were collected and boiled at 95 °C for 5 min. The samples were subjected to polyacrylamide gel electrophoresis. After electrophoresis, the gels were blotted onto PVDF membranes using Bio-Rad Trans-Blot Turbo. The membranes were blocked with 3% bovine serum albumin in TBST buffer (Tween-20 and Tris-buffered saline) for 1 h at room temperature and then incubated overnight with primary antibodies against p38 MAPK, STAT-1 and p53 at 1:2000 (Thermo Fisher, USA). The membranes were then washed with TBST buffer and incubated with HRP-conjugated secondary antibodies for 1 h at room temperature (goat anti-rabbit IgG-HRP-Img goat mAb, Novus Biologicals, USA). Bands were visualized using Clarity™ Western ECL substrate (Bio-Rad, USA cat#170–5060), and the intensity of the bands was assessed against that of β-actin with the image analysis software Chemi Doc MP imager (Markham Ontario L3R 8 T4 Canada) [23].

2.4.2. Detection of cyclin-dependent kinase inhibitor 1a (CDKN1a), p21, matrix metallopeptidase 9 (MMP9) and flavin-containing monooxygenases (FMO3) gene expression by RT-PCR

Total RNA was isolated from brain tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Primers specific for p21, MMP9 and FMO3 were used and are listed in Table 1. Real-time quantitative PCR was used to determine gene expression according to the instructions for Applied Biosystems version 3.1 software and SYBR Green I (StepOne™, USA). All data are expressed relative to the β-actin gene.

2.4.3. Determination of brain SOD, AChE, MPO and LDH activities, glutamate and 8-isoprostane levels

SOD enzyme activity was measured using a colourimetric kit (Biodiagnostic, Giza, Egypt) according to the manufacturer's instructions. AChE activity was assayed using a colourimetric kit (QuantiChrom™ Acetylcholinesterase Assay, DACE-100, CA, USA). MPO activity was assessed using a Bioassay fluorometric kit (EnzyFluo ™ Myeloperoxidase Assay, EMPO-100, CA, USA). As a marker of lipid peroxidation, 8-isoprostane levels were determined using an 8-epi-PGF2α (8-Epi-Prostaglandin F2α) ELISA kit (Elabscience, Cat#*E*-EL-0041, USA) according to the manufacturer's protocol. The enzyme activity of LDH was estimated in accordance with the protocol supplied by BioMed Diagnostic (Hannover, Germany). Glutamate levels in the brain

Table 1
Primers specific for the p21, MMP9 and FMO3 genes.

Gene	Primers
p21	Forward; 5′-TGGAGCCCCTGAAGAAGAG-3′
	Reverse; 5′-AAGTGCGTTGTGCGGTAGC-3′
MMP9	Forward; 5′-GGCGGGGTCACTGATAACGTTTTACTGCCTCT-3′
	Reverse; 5′-GCCTCCCCTCCAGGCTTATGCTGACTCA-3′
FMO3	Forward; 5′-GTTGAGGATGGCCAGGCATC-3′
	Reverse; 5′-CAGGCGTGGGTCAGTCAGGA-3′
β-actin	Forward; 5′-GGTCGGTGTGAACGGATTTGG-3′
	Reverse; 5′-ATGTAGGCCATGAGGTCCACC-3′

were estimated using an ELISA kit (MyBiosource, Cat#MBS047402, CA, USA) according to the manufacturer's instructions.

2.5. Histological analysis

The brain and spinal cord tissues from three animals of each group were collected, washed with water and immersed immediately in 10% neutral buffered formalin. Samples from the brain and spinal cord were processed routinely according to the paraffin technique. The samples were first dehydrated with ascending grades of ethyl alcohols, cleared in xylene, embedded in soft paraffin, and then embedded and blocked in hard paraffin. Sections cut at 4–6 μm thickness were used and mounted on clean and dry glass slides. The obtained slides were stained with haematoxylin and eosin (H&E) as a general stain. All techniques and stains were performed according to [24].

2.6. Statistical analysis

All results are expressed as arithmetic means ± standard error (SE). The data were evaluated statistically using the SPSS 20 (SPSS, Chicago, Ill, USA). The significance of differences among testing groups was analysed using one-way ANOVA followed by Tukey's post hoc test. Values of $p < 0.05$ were regarded as significant.

3. Results

3.1. Protein expression of p38 MAPK, p53 and STAT-1 in brain tissues

As shown in Fig. 1, the expression of p38 MAPK, p53 and STAT-1 in the brain tissues was significantly higher in the CP group than in the normal control group ($p < 0.05$). Compared to CP treatment alone, simultaneous treatment with either TQ or Ger plus CP significantly decreased the expression of p38 MAPK, p53 and STAT-1 ($p < 0.05$).

3.2. Gene expression of p21, MMP9 and FMO3 in brain tissues

The data presented in Table 2 show the levels of p21, FMO3 and MMP9 in the brain tissues of the control and test groups. Compared with the normal control group, the CP group had significantly increased ($p < 0.05$) p21, MMP9 and FMO3 levels. Simultaneous treatment with either TQ or Ger and CP significantly reduced the expression levels of only p21 and MMP9; FMO3 levels were significantly decreased only in case of TQ-treated group when compared to those of the CP-treated group ($p < 0.05$).

3.3. SOD activity

In the present study, we evaluated SOD activity in brain tissues because antioxidant enzymes can defend against oxidative stress. SOD activity was profoundly decreased in the CP group compared to that in the control group ($p < 0.05$). Simultaneous treatment with either TQ or Ger plus CP significantly prevented the CP-induced decrease in SOD activity ($p < 0.05$) (Table 3)

3.4. Brain AChE activity

AChE activity in the brain tissue was significantly decreased in the CP group compared to that in the control group ($p < 0.05$). Compared with CP treatment alone, simultaneous treatment with only CP and TQ significantly increased AChE activity, while Ger insignificantly

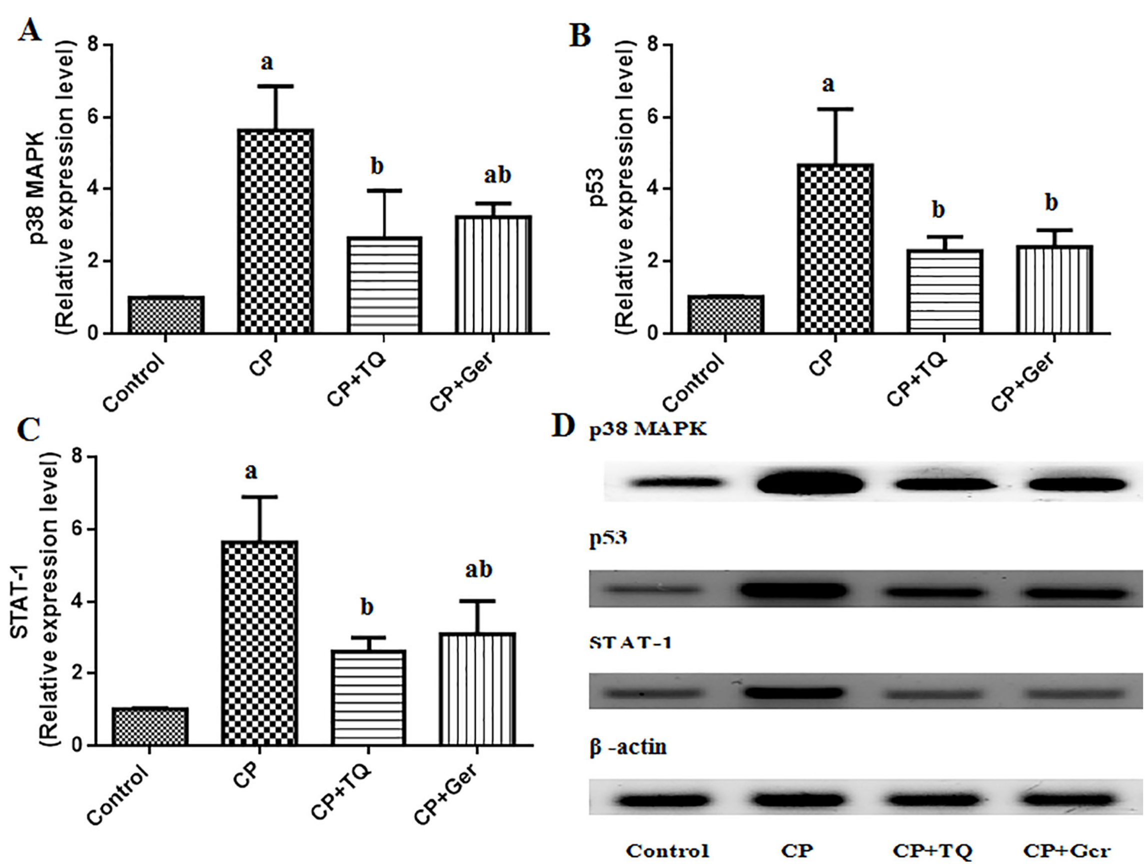

Fig. 1. Mean values of p38 MAPK, p53 and STAT-1 in the brain tissues from groups receiving simultaneous treatment with either TQ or Ger and CP or CP treatment alone. (A), p38 MAPK (B), p53 (C), STAT-1 (D), Western blot analysis showing the protein expression of p38 MAPK, p53 and STAT-1 in the brain tissues compared to that of β-actin. The data are presented as the mean ± SE, and each group consisted of 10 rats. $^{a}p < 0.05$, significant difference when compared with the control group. $^{b}p < 0.05$, significant difference when compared with the CP group.

Table 2
PCR analysis for brain p21, MMP9 and FMO3 genes expression in the different groups.

	Control	CP	CP + TQ	CP + Ger
p21 (relative expression level)	1.00 ± 0.005	5.17 ± 0.47[a]	2.21 ± 0.07[a,b]	2.81 ± 0.18[a,b]
MMP9 (relative expression level)	1.00 ± 0.002	5.34 ± 0.62[a]	2.72 ± 0.63[b]	2.72 ± 0.26[b]
FMO3 (relative expression level)	1.01 ± 0.009	6.50 ± 1.11[a]	3.04 ± 0.66[b]	3.81 ± 0.39

CP, cisplatin treatment; TQ, thymoquinone treatment; Ger, geraniol treatment.
The data are presented as the mean ± SE, and each group consisted of 10 rats.
[a] $p < 0.05$, significant difference when compared with the control group.
[b] $p < 0.05$, significant difference when compared with the CP group.

increased AChE activity in the brain tissues ($p < 0.05$) (Table 3).

3.5. Brain MPO activity

MPO activity was significantly increased in the CP group compared to that in the control group ($p < 0.05$). However, the CP-induced increase in MPO activity was significantly attenuated after simultaneous treatment with either TQ or Ger ($p < 0.05$) (Table 3).

3.6. Brain 8-isoprostane levels

To determine the effect of CP-induced oxidative stress, we measured 8-isoprostane levels. 8-isoprostane levels in the brain tissues were significantly ($p < 0.05$) higher in CP-treated rats than in normal control rats. The present results showed that simultaneous treatment with TQ or Ger plus CP decreased the lipid peroxidation and 8-isoprostane levels induced by CP administration, but treatment with Ger only significantly decreased the 8-isoprostane levels when compared to CP group ($p < 0.05$) (Table 3).

3.7. Brain function markers (glutamate levels and LDH activity)

Glutamate levels were significantly higher in rats treated with CP than in normal control rats ($p < 0.05$). Simultaneous treatment with either TQ or Ger and CP significantly decreased the glutamate levels ($p < 0.05$). The LDH enzyme activity also reflects the health status of the brain, and LDH was used as a brain function marker. LDH activity was significantly higher in CP-treated group than in the normal control group ($p < 0.05$). Simultaneous treatment with either TQ or Ger plus CP significantly reduced the LDH activity in the brain ($p < 0.05$) (Table 3).

3.8. Histopathological examination

Histopathological examination of cerebellar cortex of the rat brains (Fig. 2) showed that group 1 (normal control) had a normal cerebellar cortex with well-demarcated cortical layers (A & E), group 2 (treated with CP) had a cerebellar cortex with well-demarcated congested blood vessels and prominent oedema, severe degenerative changes in the Purkinje cells (B & F), group 3 (treated with CP + TQ) had a normal cerebellar cortex with well-demarcated cortical layers showing a normal cellular arrangement of the cerebellar cortex with normal Purkinje cells (C & G) and group 4 (treated with CP + Ger) also showed a normal cerebellar cortex with well-demarcated cortical layers, a normal cellular arrangement of the cerebellar cortex with fewer normal Purkinje cells and more degenerated Purkinje cells (D & H). Histopathological examination of the rat cerebral tissues (Fig. 3) showed that group 1 (normal control) had normal cerebral tissue with normal neurons and neuroglia (A & E), group 2 (treated with CP) had a cerebral tissue with congested blood capillaries and severe gliosis and degeneration of the nerve cells (B & F), group 3 (treated with CP + TQ) had normal and degenerated nerve cells and neuroglia (C & G) and group 4 (treated with CP + Ger) also showed normal nerve cells and neuroglia cells and degenerated neurons with pericellular oedema (D & H). Histopathological examination of the rat spinal cords (Fig. 4) showed that group 1 (normal control) had a spinal cord contained normal multipolar neurons and neuroglia (A), group 2 (treated with CP) had a spinal cord contained degenerated multipolar neurons and neuroglia (B), group 3 (treated with CP + TQ) had a spinal cord contained both normal and degenerated multipolar neurons and neuroglia (C) and group 4 (treated with CP + Ger) also had a spinal cord contained both normal and degenerated multipolar neurons and neuroglia (D).

4. Discussion

Although CP is a potent chemotherapeutic agent, its use is limited due to the associated adverse effects, including nephrotoxicity and neurotoxicity, which are major dose-limiting factors. The exact mechanism of CP-induced neurotoxicity is unclear, but there are several pathophysiological mechanisms that explain CP neurotoxicity, such as oxidative and nitrosative stress, DNA damage, apoptosis, inflammation, necrosis and mitochondrial dysfunction [25,26].

Cisplatin activates the c-Ab1 signalling pathway, resulting in the activation of the p38 MAPK pathway, which activates cell death through p53 induction [27]. p53 is a tumour suppressor protein that regulates the transcription of other genes that mediate several responses, including cell cycle arrest, DNA damage and apoptosis [28]. In response to multiple stimuli, p38 MAPK activates p53 through

Table 3
Biochemical changes in the different groups.

	Control	CP	CP + TQ	CP + Ger
SOD (U/g tissue)	6.01 ± 0.72	1.33 ± 0.25[a]	4.40 ± 0.61[b]	3.18 ± 0.30[b]
AChE (U/l)	34.76 ± 4.1	17.92 ± 1.7[a]	33.01 ± 2.05[b]	30.58 ± 1.9
MPO (U/l)	25.10 ± 2.4	74.37 ± 6.7[a]	45.17 ± 2.9[b]	35.92 ± 4.6[b]
8-isoprostane (pg/ml)	24.62 ± 2.4	83.38 ± 6.7[a]	47.37 ± 3.8[a]	42.46 ± 4.6[b]
Glutamate (μg/ml)	40.27 ± 3.13	101.13 ± 2.82[a]	73.40 ± 3.26[a,b]	75.23 ± 3.55[a,b]
LDH (U/l)	134.76 ± 4.8	321.61 ± 7.3[a]	202.42 ± 13.4[a,b]	185.37 ± 5.9[a,b]

CP, cisplatin treatment: TQ, thymoquinone treatment: Ger, geraniol treatment.
The data are presented as the mean ± SE, and each group consists of 10 rats.
[a] $p < 0.05$, significant difference when compared with the control group.
[b] $p < 0.05$, significant difference when compared with the CP group.

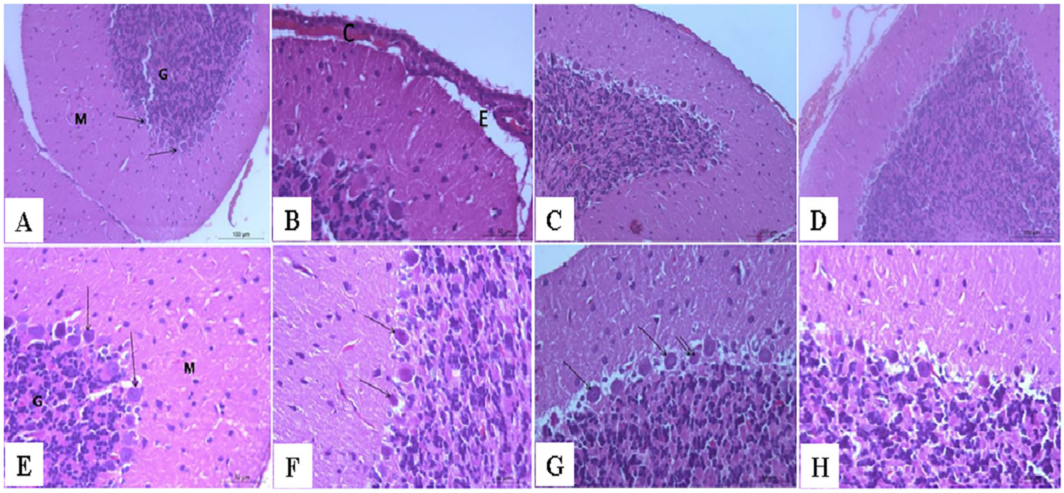

Fig. 2. Effects of TQ or Ger on cerebellar cortex histopathological changes in different studied groups. (A) Brain tissues of the normal control group showing a normal cerebellar cortex outer molecular layer [middle (M)], Purkinje layer (arrows) and inner granular layer (G), H&E stain X100, (E) higher magnification of (A), H&E stain X400. (B) Brain tissues of CP-treated group showing the cerebellar cortex with congested blood vessels (C) and prominent oedema (E), H&E stain X100, with degenerative Purkinje cells (arrows), (F) higher magnification of (B), H&E stain X400. (C) CP + TQ-treated group, brain tissues showing a normal cerebellar cortex, H &E stain X100, normal Purkinje cells (arrows) and degenerated Purkinje cells (double arrows), (G) higher magnification of (C), H&E stain X400. (D) CP + Ger-treated group, brain tissues showing a normal cerebellar cortex, H&E stain X100, (H) higher magnification of (D), H&E stain X400.

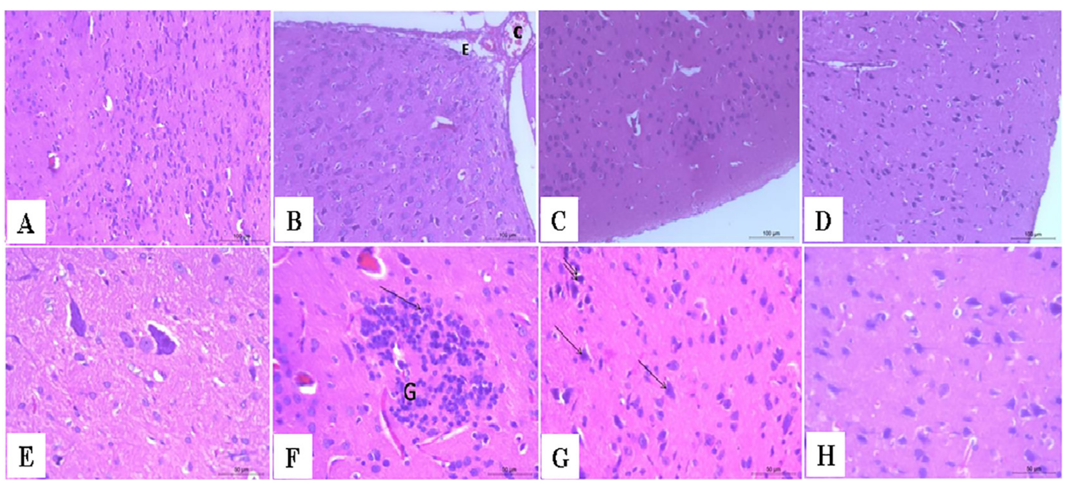

Fig. 3. Effects of TQ or Ger on cerebral tissue histopathological changes in different studied groups. (A) Brain tissues of the normal control group showing normal cerebral tissue, H&E stain X100, (E) higher magnification of (A), H&E stain X400. (B) Brain tissues of CP-treated group showing cerebral tissue with congestion (C) and oedema (E), H&E stain X100, (F) higher magnification of (B), H&E stain X400. (C) Brain tissues of CP + TQ-treated group showing cerebral tissue with normal nerve cells and neuroglia cells and degenerated neurons, H&E stain X100, (G) higher magnification of (C), neuroglia cells (arrows) and degenerated neurons (double arrows) H&E stain X400. (D) CP + Ger-treated group, brain tissues showing cerebral tissue with normal and degenerated nerve cells and neuroglia, H&E stain X100, (H) higher magnification of (D), H&E stain X400.

phosphorylating serine/threonine residues in p53 [29]. The present study showed that CP administration significantly increased p38 MAPK and p53 levels in brain tissues compared to those in the normal control group. These data were in accordance with Sahu et al. [30], who noted that the administration of CP resulted in a prominent increase in p38 MAPK and p53 in kidney tissues, exacerbating its toxic effects. Signal transducers and activators of transcription (STAT-1) proteins are transcription factors activated by cellular stress, cytokines and growth factors, and they play an essential role in cell death in response to cellular stress [31]. The cell cycle regulator p53, in coordination with STAT-1, mediates cell apoptosis as a result of CP administration [32]. Throughout our experiments, we noted that CP significantly induced STAT-1 expression. These data are in agreement with those of Schmitt et al. [33], who reported that CP-induced ototoxicity through STAT-1 activation resulted in hair cell death.

Cisplatin neurotoxicity is associated with alterations in the regulation of several genes, demonstrating the effect of CP on the peripheral nervous system through apoptosis, neurodegeneration and inflammation. MMP9 is a class of metallopeptidase family enzymes that play an important role in the maintenance and repair of tissues in the nervous system, and its expression in the neuron and glial cells is increased in response to various neuropathologies [34]. In fact, MMP9 was significantly increased in the current study in CP-treated rats. We also found that CP significantly upregulated FMO3 expression. The FMO3 gene, whose product belongs to a class of drug metabolizing enzymes, is responsible for the excretion of heteroatom-containing chemicals and their conversion into polar molecules to be excreted [35]. p21 is an important gene activated in response to CP administration in rats; this gene is a member of the Cip/Kip family that inhibits cyclin-dependent kinase (CDK), leading to cell cycle arrest, and its transcription is regulated by p53, which is linked to drug toxicity and cellular DNA damage [36]. In the current study, the expression of p21

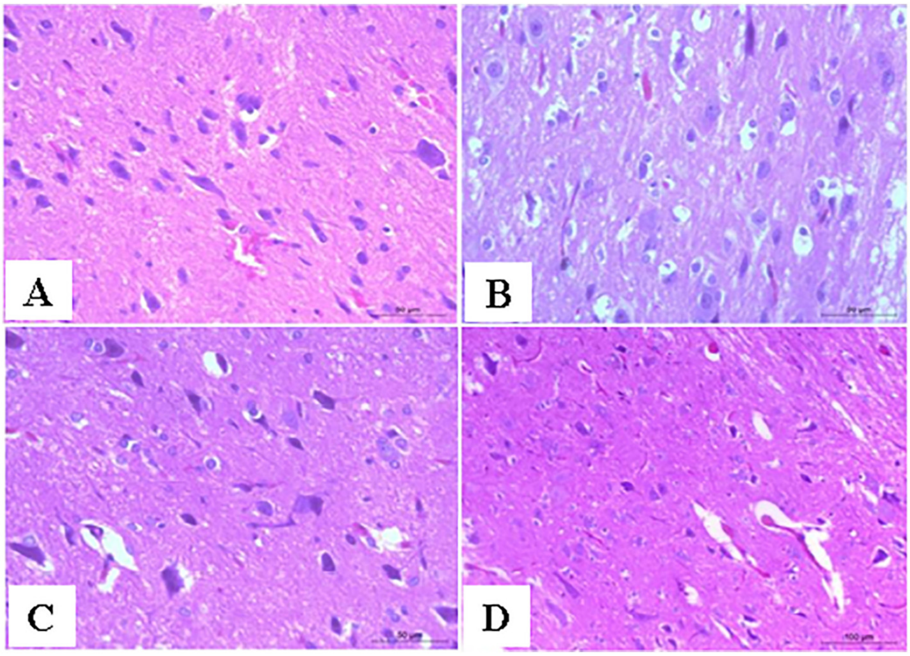

Fig. 4. Effects of TQ or Ger on spinal cord histopathological changes in different studied groups. (A) Spinal cord of the normal control group showing normal multipolar neurons and neuroglia. H&E stain X400. (B) Spinal cord of CP-treated group showing degenerated multipolar neurons and neuroglia. Note, the pericellular oedema and congestion, H&E stain X400. (C) Spinal cord of CP + TQ-treated group showing both normal and degenerated multipolar neurons and neuroglia, H&E stain X400. (D) Spinal cord of CP + Ger-treated group showing both normal and degenerated multipolar neurons and neuroglia, H&E stain X400.

was significantly increased in the CP-treated group. Our data were in line with the results of Alaedini et al. [37], who revealed that CP treatment resulted in cell apoptosis through the activation of several genes, such as p21, MMP9 and FMO3, in the dorsal root ganglia, which explains the inflammation, apoptosis and neurotoxic effects after CP treatment.

In the current study, we have revealed the protective effects of TQ, a major bioactive ingredient extracted from the volatile oil of *Nigella sativa* seeds, and Ger, the main active ingredient of the essential oils of ginger, lemon and palmarosa against CP-induced neurotoxicity in rats. The present study demonstrated that TQ and Ger treatment significantly attenuated CP-induced neurotoxicity through their antioxidant and anti-inflammatory effects. Our present data demonstrated that simultaneous treatment with either TQ or Ger suppressed CP-induced neurotoxicity in rats by reducing the histopathologic changes and significantly attenuating the CP-induced increases in MAPK family expression (p38 MAPK, STAT-1 and p53) in brain tissues. Furthermore, our results demonstrated that simultaneous treatment with either TQ or Ger in conjunction with CP significantly reduced the expression levels of only p21 and MMP9, while FMO3 levels were significantly decreased only in case of TQ-treated group when compared to those of the CP-treated group. Regarding Ger, Soubh et al. [38] reported that the antioxidant activity of Ger against 2,4,6-trinitrobenzene sulfonic acid-induced colitis occurred via the inhibition of lipid peroxidation, MPO and p38 MAPK, and Ger restored the total antioxidant capacity.

In the present study, CP administration stimulated the production of oxidative stress markers, such as MPO, which is the lysosomal protein most abundantly expressed in neutrophils that catalyses hydrogen peroxide to oxidize halide ions to hypochlorous acid. CP administration also significantly increased 8-isoprostane levels, which is a lipid peroxidation biomarker, and LDH activity, while it significantly decreased SOD activity, which reduces the toxic effect of superoxide radicals and detoxifies them to oxygen and hydrogen peroxide. These results were in accordance with those of Sahu et al. [39], who noted that CP administration significantly decreased renal antioxidant enzymes, such as SOD, and markedly increased MPO activity, which led to acute renal failure. Additionally, Ulu et al. [40] demonstrated significantly increased lipid peroxidation biomarkers, such as 8-isoprostane, as a result of CP-induced nephrotoxicity in rats. Moreover, our findings are in line with those of Farooqui et al. [41], who demonstrated that CP treatment significantly decreased SOD activity and markedly increased LDH activity in the intestinal mucosa. The present study showed that simultaneous treatment with TQ or Ger plus CP significantly increased SOD activity and decreased the 8-isoprostane levels induced by CP administration, but treatment with Ger only significantly lowered the 8-isoprostane levels compared to those in CP-treated animals. Our results also agree with those of Ahlatci et al. [42], who reported that TQ decreased the oxidative stress induced by many drugs because it has neuroprotective properties through its antioxidant activity and attenuation of lipid peroxidation. Simultaneous TQ or Ger treatment also significantly decreased the increase in MPO and LDH activity in the brain. These data are in agreement with previous studies performed in rat kidneys [40,41].

In the present investigation, to determine the influence of CP administration on cognitive and cholinergic functions, we measured the activity of AChE in the brain and found that CP treatment significantly decreased AChE activity, which led to a decrease in cholinergic activities. Several studies have reported that AChE activity was reduced in CP-treated rats when compared with that in normal controls [43], while others revealed that the administration of CP increased AChE activity [44]. These contradictory results may be due to differences in the method of determination of AChE in different parts of the brain. However, simultaneous treatment with TQ only significantly increased AChE activity, while simultaneous treatment with Ger only insignificantly increased AChE activity in the brain tissues compared with CP treatment alone. Furthermore, we observed a significant increase in glutamate levels in rat brains after CP administration. A direct relationship between cognitive dysfunction and GABA levels (a derivative of glutamate) has been found in the brain [45]. In agreement with our findings, Abdelkader et al. [46] reported that CP increased the glutamate content in rat hippocampus and cortex. Simultaneous treatment with either TQ or Ger and CP significantly reduced the glutamate levels in the brain.

In conclusion, the results of the current study demonstrated that simultaneous treatment with either TQ or Ger attenuated CP-induced neurotoxicity in rats. Thus, TQ and Ger are considered effective adjuvant therapies for CP that act through inhibiting the consequences of increases in p21, FMO3, MMP9, p38 MAPK, STAT-1 and p53 gene expression, glutamate levels and oxidative stress in brain tissues.

Conflict of interest

None to be declared.

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