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Effect of curcumin nanoparticles on the cisplatin-induced neurotoxicity in rat

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ABSTRACT

The present study is conducted to evaluate the neuroprotective effect of curcumin nanoparticles (CUR NP) against the neurotoxicity induced by cisplatin (CP) in rat. Rats were divided into control group that received saline solution, CP-treated rats that received a single i.p. injection of CP (12 mg/kg body wt), and CP-treated rats that received a single i.p. injection of CP (12 mg/kg body wt) followed by a daily oral administration of CUR NP (50 mg/kg body wt) for 14 days. At the end of the experiment, the motor activity of rats was evaluated by open field test. The neurochemical and histopathological changes were investigated in the cerebral cortex. A significant decrease in motor activity was observed in CP-treated rats. This was associated with a significant increase in the cortical levels of lipid peroxidation, nitric oxide, tumor necrosis factor-α, caspase-3, and acetylcholinesterase activity. However, CP induced a significant decrease in reduced glutathione levels and Na⁺, K⁺-ATPase activity. In rats treated with CP and CUR NP, no significant changes were recorded in the parameters of the open field test as compared to control. In addition, treatment with CUR NP prevented all the neurochemical changes induced by CP except the increased value of nitric oxide. CUR NP also reduced the histopathological changes induced by CP. It is clear from the present data that CUR NP could ameliorate the neurotoxic effect induced by cisplatin.

Introduction

Cisplatin (cis-DDP cis diammine dichloroplatinum (II)) (CP) is a chemotherapeutic agent used commonly in the treatment of solid tumors in various organs (Kasznicki et al. 2014). The neurotoxic effects induced by CP on mature neurons were demonstrated in the cerebellar cortex and ventral horn of adult rats (Abou-Elghait et al. 2010). CP penetrates into the brain where it reaches high concentrations in the patient’s brain (Nakagawa et al. 1996). In cross-sectional and prospective longitudinal studies conducted between 1995 and 2012, cognitive impairment was reported in 78% and 69% of breast cancer patients subjected to chemotherapy, respectively (Wefel and Schagen 2012). Numerous adverse effects have been reported after CP treatment including impairments in cognition, clarity of thought, attention, executive functioning, and information processing speed (Argyriou et al. 2011). These effects were also detected by formal neurophysiological testing carried out by Ahles and Saykin (2007). Advanced neuroimaging techniques showed structural changes in grey and white matter and regional variations in brain activity with disturbance in connectivity in patients suffering from cognitive impairment induced by chemotherapy (Simó et al. 2015).

It was shown that oxidative stress and mitochondrial dysfunction were the two major mechanisms involved in platinum-induced neurotoxicity that triggered neuronal apoptosis (Cavaletti et al. 2011). Moreover, CP induced neurotoxicity via oxidative damage, inflammation, mitochondrial dysfunction, DNA damage, and apoptosis (Brouwers et al. 2009, Kasznicki et al. 2014).

The adverse effects of CP were confirmed by electrophysiological and histopathological experiments (Melnikov et al. 2016, Akman et al. 2015) and have limited its clinical use at the desired dosage (Akman et al. 2015, Gorman et al. 1989). Moreover, these side effects could lead to dose reduction or early termination of chemotherapy that could affect patient’s life (Brouwers et al. 2009, Vichaya et al. 2015).

Curcumin is one of the natural products that have been extensively studied in recent years. Curcumin’s neuroprotective effect was investigated in a number of animal models of neurodegenerative diseases including epilepsy (Ezz et al. 2011), Parkinson’s disease (Wang et al. 2017), depression (Kulkarni et al. 2008), and Alzheimer’s disease (Morales et al. 2017). In addition, several studies have shown that curcumin possesses antioxidant properties, anti-inflammatory, and antioxidant effects (Kulkarni et al. 2011). However, curcumin in its natural form is characterized by low water solubility which reduces its bioavailability (Anand et al. 2007, Modasiya and Patel 2012). The low water solubility of curcumin results in decreased absorption, extensive metabolism, and rapid systemic elimination of curcumin (Yang et al. 2007, Anand et al. 2015).
Therefore, curcumin has a very short retention time in the body (Yang et al. 2007) and hence its therapeutic efficacy is restricted. Wahlstrom and Blennow (1978) demonstrated that after oral administration of 1 g/kg of curcumin, more than 75% was excreted in feces and negligible amounts of curcumin were detected in urine. These findings indicate that a limited amount of curcumin could be absorbed to the systemic circulation. Under such conditions, several studies have been carried out to solve the problem of curcumin’s low water solubility to exploit the benefits of curcumin and exaggerate its therapeutic efficacy. One of such strategies is to use curcumin in its nanoparticle form. Curcumin nanoparticles (CUR NP) can be formulated using chitosan-alginate and sodium tripolyphosphate which enhances its aqueous solubility and ability to penetrate the blood-brain barrier (Hashemian et al. 2017). Although both curcumin and nanocurcumin could cross the blood-brain barrier, nanocurcumin has a better retention capacity in the hippocampus (increase by 83%) and cerebral cortex (increase by 96%; Tsai et al. 2011a). This property of nanocurcumin may increase its therapeutic efficacy. Furthermore, oral nanocurcumin has been found to be 22% more bioavailable than curcumin itself (Tsai et al. 2011b). It has been suggested that nanocurcumin represents a promising therapeutic advance over native curcumin (Flora et al. 2013).

The present study aims to investigate the use of CUR NP to attenuate the neurotoxicity of CP by measuring oxidative stress parameters, caspase-3, tumor necrosis factor-α (TNF-α), and the activities of acetylcholinesterase (AChE) and Na⁺, K⁺-ATPase in the cerebral cortex. In addition, the changes in motor activity and histological profile were evaluated.

**Material and methods**

**Animals**

Twenty-four adult male Wistar rats, weighing between 160 and 180 g, were obtained from the Animal House of the National Research Centre, Egypt. They were housed under temperature- and light-controlled conditions with standard laboratory rodent chow and water provided ad libitum. Animal procedures were approved by the Ethics Committee of the National Research Centre (registration No 14149) and were performed in compliance with the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

**Chemicals**

CP was purchased from Mylan Company, France. CUR NPs were obtained from the Department of Food Toxicology and Contaminants, National Research Center, Egypt where CUR NPs were prepared using a modified method of Li et al. (2015) as described by Ismaiel et al. (2015). In brief, 100 mg of curcumin (0.27 mmol) was dissolved in 20 ml dichloromethane and 1 ml of this solution was sprayed into 50 ml boiling water drop wise with a flow rate of 0.2 ml/min for 5 min under ultrasonic conditions using an ultrasonic power of 100 W and a frequency of 30 kHz. The contents were stirred at 200–800 rpm at room temperature for 20 min after sonication for 10 min to obtain a clear orange-colored solution. This solution was concentrated under reduced pressure at 50 °C and then freeze-dried to obtain an orange powder. Transmission electron microscopy (TEM) was used to determine the size of the synthesized nanocurcumin. The particles were examined using TEM (JEM-HR-2100 electron microscope, Japan) at total magnification 6.00 kx and accelerating voltage 200 kV. The produced nanoparticles were nearly spherical with an average diameter of 40 nm as shown in Figure 1 and they were characterized by high water solubility.

**Experimental design**

At the beginning of the experiment, 24 rats were divided randomly into: control rats (n = 8) that received a single intra-peritoneal (i.p) injection of saline solution (0.9%) followed by daily oral administration of saline solution (0.9%) for 14 days; CP-treated rats (n = 8) that received a single i.p. injection of CP (12 mg/kg body wt) followed by a daily oral administration of saline solution (0.9%) for 14 days and rats (n = 8) treated with a single i.p. injection of CP (12 mg/kg body wt) followed by a daily oral administration of CUR NP (50 mg/kg body wt) for 14 days.

At the end of experiment the following measurements were performed for all the experimental groups.

**Behavioral analysis**

**The open field test (OFT)**

The open field apparatus was constructed of white plywood and measured 72 × 72 cm with 36 cm walls. One of the walls was clear plexiglas, so rats could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were
visible through the clear plexiglas floor. The lines divided the floor into 16 18 × 18 cm². A central square (18 cm × 18 cm) was drawn in the middle of the open field. Rats were placed individually in the center of the open field and behavioral parameters were assessed manually for 10 min. Five motor parameters were quantified throughout this test: central square duration (the duration of time the rats spent in the central square), line crossings (the number of times the rats crossed one of the grid lines with all four paws), rearing (the number of times the rats stood on their hind legs in the open field), freezing time (duration in which the rat was completely stationary), and grooming (number of body-cleaning with paws, licking of the body and pubis with the mouth, and face-washing actions). The open field apparatus was cleaned after each session using 70% ethyl alcohol and permitted to dry between tests (Brown et al. 1999).

**Neurochemical analyses**

On the 15th day the animals were sacrificed and the cerebral cortex of each rat was dissected out, weighed and kept frozen at −80 °C. The cortex of each rat was homogenized in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 5000 rpm. and 2 °C for 10 min. The supernatant was stored at −80 °C until carrying out the following parameters.

**Determination of lipid peroxidation**

Malondialdehyde (MDA), a measure of membrane lipid peroxidation, was determined according to the method of Ruiz-Larrea et al. (1994). MDA was determined by measuring thiobarbituric reactive species. One molecule of MDA reacted with two molecules of thiobarbituric acid in acidic medium at temperature of 95 °C for 20 min to form thiobarbituric acid reactive substances; the absorbance of the resultant pink product was measured at 532 nm using UV-visible spectrophotometer (model UV-2401 PC, Shimadzu, Japan).

**Determination of nitric oxide**

Nitric oxide (NO) was determined spectrophotometrically in the brain tissue according to the method described by Montgomery and Dymock (1961). This method was based on the measurement of endogenous nitrite concentration as an indicator of nitric oxide production. It depended on the addition of Griess reagent which converted nitrite into a deep purple azo compound whose absorbance was read at 540 nm.

**Determination of reduced glutathione**

Reduced glutathione (GSH) level in the cerebral cortex was determined by the method of Beutler et al. (1963). This method was based on the reduction of 5, 5′- dithiobis-(2-nitrobenzoic acid) or DTNB "Ellman’s reagent" with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at 412 nm. Total GSH content was expressed in mmol/g brain tissue.

**Determination of caspase-3**

Caspase-3 was determined in the cortex using ELISA Kit SG-20396 supplied by SinoGeneclon Co. Ltd (China). The concentration of caspase-3 was expressed in μg/g brain tissue.

**Determination of TNF-α**

Estimation of TNF-α level in the cortex was carried out using rat TNF-α ELISA Kit SG-20127 supplied by SinoGeneclon Co. Ltd (China). The concentration of TNF-α was expressed in μg/g brain tissue.

**Determination of AChE activity**

AChE activity was measured according to the modified method of Ellman et al. (1961). The principle of the method depended on the hydrolysis of acetylthiocholine iodide by AChE to produce thiocholine. Thiocholine was allowed to react with the -SH reagent DTNB, which was reduced to thionitrobenzoic acid, a yellow colored anion whose absorption was read spectrophotometrically at 412 nm. The results were expressed as μmol SH/min/g brain tissue.

**Determination of Na⁺, K⁺-ATPase activity**

Na⁺, K⁺-ATPase activity was measured spectrophotometrically according to Tsakiris et al. (2000). Na⁺, K⁺-ATPase activity was calculated as the difference between total ATPase activity (Na⁺, K⁺-ATPase and Mg-ATPase activity) and Mg-ATPase activity.

Total ATPase activity was assayed by adding 50 μl of the brain tissue homogenate to 2.5 ml of incubation medium consisting of 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 20 mM KCl, 4 mM MgCl₂, 240 mM sucrose, 1 mM ethylenediamine tetraacetic acid, and 3 mM disodium ATP (substrate). After an incubation period of 10 min at 37 °C, the reaction was stopped by the addition of 50 μl ice cold trichloroacetic acid (30%). Then the mixture was centrifuged at 3000 rpm for 15 min. About 1 ml of the supernatant was added to 500 μl of 10% trichloroacetic acid, 250 μl ammonium molybdate (1%), and 250 μl ascorbic acid (20%) and used for the determination of the liberated inorganic phosphate (Pi). After 20 min at room temperature, the developed color was read at 640 nm against blank which contained 1500 μl of 10% trichloroacetic acid, 250 μl ammonium molybdate (1%), and 250 μl ascorbic acid (20%). The amount of liberated inorganic phosphate was quantified using KH₂PO₄ as a reference standard. The previous steps were repeated in the presence of 1 mM of ouabain (specific Na⁺/K⁺-ATPase inhibitor) in the incubation medium.

**Histological examination**

Histological studies were carried out primarily for confirmative/illustrative purposes. For assessment by means of light microscopy, the brains were fixed in 10% formaldehyde, embedded in paraffin, and cut serially at 5 μm sections. Routine staining of sections with hematoxylin–eosin (H&E) was performed. The sections were examined microscopically.
for histopathological changes such as the damaged neurons, changes in nuclei, and vacuolation inside and outside the cytoplasm. In addition, cerebral vascular congestion and inflammation were evaluated.

Statistical analysis
The data were expressed as means ± SEM. Statistical significance between the groups under investigation was tested by one-way analysis of variance using Statistical Package for Social Sciences (SPSS) program followed by Duncan as post hoc test to compare the significance between groups. The difference was considered significant at p-value < 0.05.

Results

Neurochemical results
The present data showed a significant increase in the levels of lipid peroxidation (81.07%) and nitric oxide (107.57%) in the cerebral cortex of rats treated with CP as compared to control values. This was associated with a significant decrease in reduced glutathione level. In addition, cortical TNF-α and caspase-3 increased significantly recording 30.52% and 68.63%, respectively, above the control values. When the CP-treated rats were treated daily with CUR NP for 14, the levels of lipid peroxidation (81.07%) and nitric oxide (107.57%) in treated rats were treated daily with CUR NP for 14, the levels of lipid peroxidation, reduced glutathione, caspase-3, and TNF-α returned to nearly control-like values. However, CUR NP failed to prevent the increased level of nitric oxide induced by CP (Table 1).

CP induced a significant increase in AChE activity (97.55%) and a significant decrease in Na+, K+-ATPase activity (−12.5%) compared to control rats. CUR NP prevented the changes induced in these enzymes (Table 2).

Behavioral results
CP treatment resulted in a significant increase in the time spent in central square (360%) and freezing time (99.9%) and a significant decrease in the number of crossed lines (−73.34%), number of rearings (−65.7%), and number of groomings (66.9%). These changes induced in motor activity were restored by CUR NP to control like values (Figure 2).

Histopathological results
Normal histological appearance of neurons with sharply demarcated nuclei and normal structure were observed in the cerebral cortex of control rats (Figure 3(a)). The cerebral cortex of CP-treated rats showed shrinkage of the cytoplasm and extensively dark pyknotic nuclei with chromat in condensation in neurons (Figure 3(b)). In addition, vascular congestion and cellular infiltration were also observed in the pia mater (Figures 3(d) and 2(e)). These histological changes decreased markedly when rats were treated with CUR NP. In the cerebral cortex of rats treated with CP and CUR NP, the shrunken cytoplasm and the extensively dark pyknotic nuclei decreased compared to CP-treated group. In addition, vascular congestion and cell infiltration were reduced in pia mater compared to CP-treated group (Figure 3(c) and (f)).

Discussion
The present findings showed that CP induced oxidative stress in the cerebral cortex of rat brain. This is indicated from the significant increase in the levels of MDA and NO and the decreased levels of GSH.

It has been reported that both short- and long-term administration of CP caused oxidative stress by forming reactive oxygen species (ROS) with a concomitant increase in lipid peroxidation and decline in antioxidant enzyme activity in tissues (Qu et al. 2012). The oxidative stress induced by CP in the nervous system has been attributed to the accumulation of platinum in the cerebral tissue which leads to oxidative damage and apoptotic cell death (Carozzi et al. 2010).

Taking into consideration the high content of polyunsaturated membrane lipids in the brain together with its reduced

| Table 1. Effect of daily curcumin nanoparticles (CUR NP) treatment (50 mg/kg) on the levels of lipid peroxidation (MDA) (nmol/g), nitric oxide (NO) (μmol/g), reduced glutathione (GSH) (nmol/g), caspase-3 (μg/g), and tumor necrosis factor-α (TNF-α) (μg/g) in the cortex of cisplatin-treated rats. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | Control         | Cisplatin       | %D              | Cisplatin + CUR NP | %D              | p-value         |
| MDA                            | 9.69 ± 1.936    | 17.54 ± 1.722   | 81.07           | 8.695 ± 0.954      | −10.27          | 0.001           |
| NO                             | 0.066 ± 0.007   | 0.137 ± 0.020   | 107.57          | 0.119 ± 0.001      | 80.30           | 0.016           |
| GSH                            | 8.40 ± 0.069    | 7.40 ± 0.145    | −11.85          | 8.679 ± 0.288      | 3.31            | 0.015           |
| Caspase-3                      | 16.50 ± 1.450   | 27.82 ± 5.3745  | 68.63           | 18.924 ± 0.672     | 14.68           | 0.046           |
| TNF-α                          | 115.38 ± 5.248  | 150.594 ± 8.128 | 30.52           | 122.17 ± 7.51925   | 5.88            | 0.010           |

Values represent mean ± SE with the number of animals between parentheses.
% D: % difference with respect to control values.
Different letters indicate significantly different means p-value < 0.05.
Same letters indicate nonsignificant changes.

| Table 2. Effect of daily curcumin nanoparticles (CUR NP) treatment (50 mg/kg) on the activity of acetylcholinesterase (AchE) (μmol SH/g/min) and Na, K, ATPase (μmol Pi/min/g), in the cortex of cisplatin-treated rats. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Control         | Cisplatin       | %D              | Cisplatin + CUR NP | %D              | p-value         |
| AchE                           | 1.835 ± 0.147   | 3.625 ± 0.371   | 97.55           | 2.082 ± 0.295     | 13.46           | 0.001           |
| Na,K,ATPase                    | 0.136 ± 0.002   | 0.119 ± 0.003   | −12.50          | 0.128 ± 0.004     | −5.88           | 0.035           |

Values represent mean ± SE with the number of animals between parentheses.
% D: % difference with respect to control values.
Different letters indicate significantly different means p-value < 0.05.
Same letters indicate nonsignificant changes.

Discussing the high content of polyunsaturated membrane lipids in the brain together with its reduced
antioxidant mechanism (Asha Devi 2009) and its high metabolic rate, the brain is considered one of the most vulnerable organs to oxidative stress. Mitochondrial glutathione (GSH) is essential in regulating the inner mitochondrial permeability and enzyme function by keeping the SH group in the reduced state, otherwise the SH-groups of enzymes become inactivated. The toxicity of CP is probably related to the reduction in the intracellular GSH concentration and protein bound SH-groups by decreasing nicotinamide adenine dinucleotide (NADH), which helps in maintaining these groups. The depletion of GSH and NADH seems to inhibit several dehydrogenases, causing the uncoupling of oxidative phosphorylation and leading eventually to hydroxyl radical generation and oxidative stress. In addition, the formed free radicals attack the membrane polyunsaturated lipids and proteins resulting in lipid peroxidation and damaging membrane integrity (Aggarwal 1998).

The activation of CP occurs immediately after it enters the cell. Under low intracellular chloride ion concentrations, CP is hydrolyzed giving reactive species with different charges including diaquated [cis-(NH)Pt(HO)]²⁺ and monoaqua [cis-(NH)PtCl(HO)]⁺ forms (Aggarwal et al. 1980, Jennerwein and Andrews 1995) which are 1000 times more reactive than the original CP, and inhibit mitochondrial respiration through the uncoupling of oxidative phosphorylation (Aggarwal 1993). This action results in calcium efflux from the mitochondria thereby increasing intracellular calcium levels, and disrupting normal calcium homeostasis, and thus cellular functions (Dasari and Tchounwou 2014). Therefore, the cortical increase in lipid peroxidation induced by CP, in the present study, may arise from increased ROS and reactive nitrogen species (RNS) which could attack the phospholipids in the membranes. Furthermore, the present decreased GSH level may be due to its expenditure as a scavenger for the developed free radicals.

The increased intracellular calcium level can initiate a cascade of events that promote free radicals generation, cytochrome c release from mitochondria, and activation of caspases, leading to apoptotic cell death (Ferreiro et al. 2006). Thus, the increased intracellular calcium level induced by CP could underlie the present increase in caspase-3.

It is now well established that conditions that promote oxidative/nitrosative stress result in excessive Ca²⁺ release that can provoke pathological responses and neuronal death (Kishida and Klann 2007).

TNF-α is a cytokine produced from astrocytes, neurons (Gahring et al. 1996), and microglia, the latter being considered its major source during neuroinflammation (Welser-Alves and Milner 2013). In pathological conditions, large
amounts of TNF-α are released from astrocytes and mainly from microglia; this de novo synthesis of TNF-α is a chief component of the neuroinflammatory response correlated with several neurodegenerative disorders (Montgomery and Bowers 2012). Moreover, this cytokine can stimulate glutamate-induced cytotoxicity (Pickering et al. 2005) which leads to a massive influx of calcium ions inside the neurons with the production of ROS and RNS and neuronal death (Dong et al. 2009). Accordingly, the present increase in cortical TNF-α level contributes to the state of neurotoxicity induced by CP through glutamate excitotoxicity that exaggerates the oxidative damage.

Na⁺, K⁺-ATPase activity acts to restore the membrane action potential by the efflux of Na ions and influx of K ions (Shytle et al. 2002). Therefore, decreased Na⁺, K⁺-ATPase activity has been linked with increased excitability (Souza et al. 2013). The present decrease in cortical Na⁺, K⁺-ATPase activity induced by CP may be a consequence of the excitotoxicity mediated by increased TNF-α. Vasić et al. (2008) reported that CP inhibited Na⁺, K⁺-ATPase activity and this effect was ascribed to the formation of CP-ATPase complex. The authors suggested that the recovery of the enzyme activity could be achieved by legends containing thiol group such as reduced glutathione and l-cysteine. Thus, the present decreased level of cortical GSH may also contribute to the inhibition of Na⁺, K⁺-ATPase activity induced by CP. The decreased Na⁺, K⁺-ATPase activity could exacerbate the state of excitotoxicity.

AChE plays a key role in cholinergic transmission by catalyzing the rapid hydrolysis of the neurotransmitter

![Figure 3. Effect of daily curcumin nanoparticles (CUR NP) treatment (50 mg/kg) on the histopathological changes induced by cisplatin in the cortex of rats (H&E × 400). (a) Section of the cortex of control rat showing normal neurons indicated by black arrows. (b) Section of the cortex of cisplatin-treated rats showing shrinkage of the cytoplasm and extensively dark pyknotic nuclei in neurons indicated by red arrows. (c) Section of the cerebral cortex of rats treated with CUR NP and cisplatin showing normal neurons as indicated by black arrows and a decrease in the number of dark pyknotic nuclei and shrinkage of the cytoplasm indicated by red arrows. (d) Section of the cerebral tissue of cisplatin-treated rats showing vascular congestion and inflammatory infiltration in pia matter indicated by red arrows. (e) Section of the cerebral cortex of cisplatin-treated rats showing vascular congestion and cell infiltration indicated by red arrows. (f) Section of the cerebral cortex of CUR NP and cisplatin-treated rats showing reduced vascular congestion and cell infiltration indicated by black arrow in pia matter compared to cisplatin group.](image-url)
acetylcholine (Gabrovaska et al. 2008). Our present results are in agreement with the study of Owoeye et al. (2018) who found that CP induced an increase in AChE activity. Therefore, the increased cortical activity of AChE could decrease the cholinergic activity. This effect may mediate the reduced cognitive function and memory induced by CP.

It is clear from the histological examination that CP induced shrinkage of cytoplasm, extensively dark pyknotic nuclei in neurons of cerebral cortex, vascular congestion, and cellular infiltration in the cerebral cortex. These effects could be explained by the oxidative damage induced by ROS and RNS, neuroinflammation mediated by increased TNF-α and apoptosis caused by the increased level of caspase-3. Apoptosis is considered as an energy-dependent controlled type of cell death which leads to chromatin condensation, cell shrinkage, membrane budding, phosphatidylserine externalization, and the activation of caspases (Salvesen and Dixit 1997). Caspase activation is the initial and key step in apoptosis. Caspases may act either as initiators or executioners of apoptotic cell death. Activation of initiator caspases, as caspases 8 and 9, results in activation of executioner caspases as caspases 3 and 7 (Salvesen and Abrams 2004). Thus, the present increased level of cortical caspase-3 may mediate the neuronal cell death induced by CP.

The behavioral data demonstrate that CP reduces the motor activity of rats as indicated from the significant increase in the time elapsed in the central square and freezing time and the significant decrease in number of crossed lines and number of rearings. Our results are in parallel with the findings of Ali et al. (2014) who reported that CP treatment significantly decreased motor and exploratory activities, and increased immobility time in depression models, suggesting a possible depressive-like state. More recently, Abdelkader et al. (2017) have shown that CP increased immobility time and reduced the number of crossed squares and number of rearings. They attributed the decrease in motor activity to the depressive-like symptoms induced by CP. These data are in harmony with previous studies that showed that CP administration impaired explorative behaviors, memory retention, and locomotor activity in rats (Golchin et al. 2015). Therefore, the hypoactivity induced by CP in the present study may reflect the development of depressive symptoms.

Curcumin is a natural product that has antioxidant and anti-inflammatory activities and could prevent cell death. These effects have been reported in different animal models of neurodegenerative diseases. In addition, it has been reported that the curcumin molecule possessed antitumor properties but, unlike other known chemotherapeutic compounds, it did not cause any damage to the normal cells (Syng-Ai et al. 2004). However, the low water solubility of curcumin results in limited bioavailability and hence reduced efficacy. Yang et al. (2012) reported 1% bioavailability for oral administration of curcumin in rats. Due to its rapid intestinal and hepatic metabolism, about 60–70% of an oral dose of curcumin gets eliminated by feces (Pan et al. 1999). This challenge has been resolved by using curcumin in nanoform which is characterized by high water solubility and increased bioavailability. This effect could increase the retention time of curcumin in the tissues. As a result the therapeutic efficacy of curcumin will be increased.

The present data revealed that CUR NP ameliorates the oxidative stress induced by CP in the cerebral cortex. CUR NP alleviated the changes in cortical lipid peroxidation and GSH levels resulting from CP. Curcumin acts directly and indirectly as antioxidant. It can scavenge ROS such as hydrogen peroxide, peroxyl radicals, superoxide anion, hydroxyl radicals, singlet oxygen, and RNS such as nitric oxide, and peroxynitrite anion (Barzegar and Moosavi-Movahedi 2011). In addition, curcumin could stimulate the synthesis of GSH (Biswas et al. 2005). Therefore, the antioxidant activity of CUR NP that was observed in the present study could be attributed not only to curcumin’s scavenging activity but also to its enhancement of the antioxidant mechanisms such as reduced glutathione. The ability of CUR NP to scavenge ROS and RNS arising from CP treatment will prevent these radicals from attacking the phospholipids and this may explain the control-like value of lipid peroxidation and GSH induced in cortex of CUR NP-treated rats.

The present findings showed also that CUR NP prevented the elevated level of TNF-α and caspase-3 induced by CP to nonsignificant changes as compared to control values. Curcumin has been shown to down-regulate TNF-α expression (Lee et al. 2007). This effect may in turn prevent the TNF-α-induced excitotoxicity mediated by glutamate. Supporting this explanation is the control-like activity of Na⁺, K⁺-ATPase that was observed in CUR NP-treated rats. Thus, CUR NP could prevent the subsequent events; calcium influx, free radical production, and neuronal cell death that follow glutamate excitotoxicity induced by CP. This action may prevent the neuronal damage induced by CP. It may also explain the present CUR NP-induced reduction in caspase-3, an autocatalytic enzyme, which is the major deleterious caspase involved in apoptosis (Nicholson et al. 1995). These findings agree with the study of Bo et al. (2017) who found that curcumin could reduce cell apoptosis by reducing the expression of caspase-3, p53, and bax/bcl2 ratio.

The antioxidant and anti-inflammatory activities of CUR NP may mediate the improvement in the histological changes induced by CP in the cortex as evident from the reduced shrinkage of cytoplasm and pyknotic nuclei to a large extent in CUR NP-treated rats. In addition, CUR NP decreased vascular congestion and cell infiltration in pia matter as compared to CP. Curcumin was also found to reduce pathogenesis of CaCl₂-induced cerebral aneurysm of brain cells through inhibition of mitochondrial apoptosis process, and cerebral aneurysm is a cerebrovascular disorder in which weakness in the wall of a cerebral artery and vein causes ballooning of the blood vessel (Bo et al. 2017). Therefore, the antiapoptotic effect of CUR NP could contribute to the reduction of vascular congestion and cell infiltration induced in the cerebral tissues by CP.

The present results showed also that CUR NP normalized cortical AChE activity. Numerous studies have shown that central cholinergic system plays an important role in cognitive functions, and drugs affecting this system have been shown to change performance in tests of learning and memory (Kruk et al. 2011). Therefore, the restoration of cortical
AChe may help in achieving a normal level of acetylcholine. This effect may prevent the decline in cognitive function reported after CP.

The present treatment with CUR NP prevented the behavioral changes induced by CP. In our previous work, we have observed that the reduction in motor activity was linked to the reduction in the levels of serotonin, dopamine, and norepinephrine in rat model of depression (Khadrawy et al. 2017). Several studies found that curcumin possesses an antidepressant effect mediated by increasing the levels of serotonin, norepinephrine, and dopamine (Kulkarni et al. 2008, Lopresti and Drummond 2017). Thus, the present improved motor activity may indicate the ability of CUR NP to prevent despair and depressive symptoms.

Conclusions

In conclusion, the present findings suggest that CUR NPs could efficiently ameliorate the neurotoxicity induced by CP. This was mediated by the potential antioxidant, anti-inflammatory, and anti-apoptotic effects of CUR NPs. These effects were reflected in the ability of curcumin to improve the histopathological changes caused by CP in the cerebral cortex.

Therefore, CUR NPs could not only prevent the adverse effects of CP but could also act as an antitumor agent. Accordingly, CUR NPs could be used with CP to prevent its toxicity and increase its efficiency as an antitumor. This in turn may help in reducing the dose of CP without affecting its potency.

Disclosure statement

No potential conflict of interest was reported by the authors.

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