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RESEARCH ARTICLE

Protective effects of *Curcuma longa* against neurobehavioral and neurochemical damage caused by cerium chloride in mice

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Abstract
Cerium chloride ($CeCl_3$) is considered an environmental pollutant and a potent neurotoxic agent. Medicinal plants have many bioactive compounds that provide protection against damage caused by such pollutants. *Curcuma longa* is a bioactive compound-rich plant with very important antioxidant properties. To study the preventive and healing effects of *Curcuma longa* on cerium-damaged mouse brains, we intraperitoneally injected cerium chloride ($CeCl_3$, 20 mg/kg BW) along with *Curcuma longa* extract, administrated by gavage (100 mg/kg BW), into mice for 60 days. We then examined mouse behavior, brain tissue damage, and brain oxidative stress parameters. Our results revealed a significant modification in the behavior of the $CeCl_3$-treated mice. In addition, $CeCl_3$ induced a significant increment in lipid peroxidation, carbonyl protein (PCO), and advanced oxidation protein product levels, as well as a significant reduction in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Acetylcholinesterase (AChE) activity remarkably increased in the brain of $CeCl_3$-treated mice. Histopathological observations confirmed these results. *Curcuma longa* attenuated $CeCl_3$-induced oxidative stress and increased the activities of antioxidant enzymes. It also decreased AChE activity in the $CeCl_3$-damaged mouse brain that was confirmed by histopathology. In conclusion, this study suggests that *Curcuma longa* has a neuroprotective effect against $CeCl_3$-induced damage in the brain.

Keywords Cerium chloride · Neurotoxicity · *Curcuma longa* · Oxidative stress · Mice

Introduction

Cerium (Ce) is a member of the lanthanides, known as the rare earth elements. Ce is a silvery-white heavy metal. It is present in nature in different oxidized forms, most commonly the trivalent Ce3+ and tetravalent Ce4+ states (Reinhardt and Winkler 2002; Kilbourn 2003). It is used in various sectors such as agriculture, industry, and medicine, as well as in everyday life, and its amount and diversity of uses are increasing annually (Vinod et al. 2006). The accumulation of this metal in the environment is transferred to humans through the food chain. Recent studies have shown that Ce toxicity is involved in several liver and kidney dysfunctions (Beltifa et al. 2017). The central nervous system (CNS) is one of the organs most impaired by Ce exposure (Zhao et al. 2011). The brain has a high metabolic rate, a reduced capacity for cell regeneration (low levels of endogenous scavengers, vitamin C, catalase, superoxide dismutase (SOD)), and numerous cellular oxidative stress targets (lipids, nucleic acids, and proteins), which makes it the most vulnerable organ to oxidative stress (Cui et al. 2004; Zhao et al. 2010). Oxidative stress is created by the accumulation of reactive oxygen species (ROS), lipid peroxidation, and decrease in antioxidant defense (enzymatic or non-enzymatic) (Ilhami 2012). Cui et al. (2004) showed that oxidative stress causes several neurodegenerative diseases. Neurodegeneration is strongly linked to animal behavior and memory. In this context, a study conducted by Zhao et al. (2012) showed that chronic exposure to $CeCl_3$ in mice decreases memory and learning ability.

Many studies have investigated means to prevent or treat damage caused by environmental pollutants. There is increasing evidence in the literature that a diet rich in polyphenols

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may prevent or counter the harmful effects of heavy metals. Furthermore, several studies have provided evidence for the medicinal properties of turmeric (*Curcuma longa*) and its health benefits (Osawa et al. 1995).

Curcuma longa is a tropical plant of the family Zingiberaceae native to Southeast Asia (Záveská et al. 2016) Most active molecules in this plant are located in the rhizome, the most important of which is curcumin. Curcumin is a polyphenolic molecule and its chemical nomenclature is 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or more concisely diferuloymethane (Tuba Ak İlhami 2008). Tønnesen and Karlsen (1986) and Sharma et al. (2004) showed that in neutral and acidic pH, curcumin acts as a proton donor and in a basic pH as an electron donor; this is considered the origin of its antioxidant properties.

Nowadays, curcumin is a known medicinal product, thanks to its antioxidant, anti-inflammatory, anticancerous, and antibiotic properties (Kohli et al. 2005; Garcea et al. 2005; Nwozo et al. 2009; Sethi et al. 2009).

To our knowledge, no investigation has reported so far on the protective effects of *Curcuma longa* against $CeCl_3$ neurotoxicity in adult mice. The aim of our study was to explore the effects of $CeCl_3$ on mouse behavior, oxidative stress, and histopathological changes in the brain, and the efficacy of *Curcuma longa* administration in preventing brain damage induced by $CeCl_3$.

Materials and methods

Chemicals

$CeCl_3$ and all other chemicals, used in biochemical assays, were purchased from Sigma Chemicals Co. (St. Louis, France).

Plant material

The rhizomes of turmeric longa were purchased from the market. Professor Ferjani Ben Abdallah, a botanist in the Department of Life Sciences, Faculty of Science of Sfax, Tunisia, has authenticated the plants as *Curcuma longa*.

Extraction procedure

The *Curcuma longa* rhizomes were ground and the resulting powder was kept at 4 °C until use. The extraction was done by maceration. The macerates were filtered by a Whatman filter. Then, the resulting solutions were evaporated using a rotary evaporator.

To extract curcumin, we used ethanol and methanol as organic solvents according to Boullay et al. (2013) (Table 1). The extracts were lyophilized and kept at 4 °C until further use.

Table 1 Different conditions of extraction of *Curcuma longa*

	Temperature (°C)	Ratio	rpm	Time
Methanol 96%	37	1:6	125	24
Ethanol 96%	37C	1:6	125	24
Ethanol 70%	80	1:6	30	12
Ethanol 70%	37	1:6	125	24

Phytochemical analysis of the plant extracts

Determination of total phenolic and flavonoid contents

As described by Li et al. (2008), the total phenolic content was quantified at 760 nm. The total phenolic content was expressed in milligrams of gallic acid (GAE) equivalents per gram of plant extract (mg GAE/g).

The flavonoid content was analyzed as per Quettier-Deleu et al. (2000). The absorbance of the samples was measured at 430 nm and expressed in milligram of quercetin (QE) equivalent per gram of plant extract (mg QE/g).

Antioxidant testing assays

DPPH free radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was determined as per Barros et al. (2007). Butylated hydroxytoluene (BHT) was used as a positive control. The absorbance was read in 517 nm after 30 min of incubation in obscurity (Topal et al. 2016). The reduction percentage of DPPH was calculated according to the following equation:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A0-A1}{A0} \times 100$$

The results were expressed as IC_{50} (inhibition level was 50% DPPH radical).

TAA

For total antioxidant activity (TAA), the total antioxidant capacity of the extracts was evaluated as per Prieto et al. (1999). Briefly, 0.1 ml of sample was mixed with 3 ml (sulfuric acid 0.6 M, 28 mM sodium phosphate buffer and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. Absorbance was measured at 695 nm against a blank.

Animals and treatment

Our study was performed on 24 adult male mice (30 ± 5 g, 6 weeks). Those mice were randomly divided into four groups of 8 each:

- Control: given food and tap water ad libitum
- $CeCl_3$: injected intraperitoneally with cerium chloride (20 mg/kg BW) (Zhao et al. 2011)
- $CeCl_3$+Curcuma.L: in this group, $CeCl_3$ injection (20 mg/kg BW) was accompanied with extract of *Curcuma longa* (ECL) by gavage (100 mg/kg BW)
- Curcuma.L: received extract of *Curcuma longa* (ECL) by gavage (100 mg/kg BW)

The treatments were carried out for 60 days. During the experimental period, mice were housed at room temperature 22 ± 3 °C with alternating 14 h of light and 10 h of darkness with a standard diet and free access to tap water.

Behavioral tests

After 30 days of treatment, open field and radial 8-arm maze (RAM) tests were performed. Tests lasted for 15 days.

The OF test As per Sethi et al. (2008) and Allagui et al. (2014), the open field (OF) test is used to assess the general locomotor activity of mice or rats. The test is performed in a rectangular roofless box with a floor divided into identical squares. A mouse is placed in the center of the apparatus and is let free for 5 min. During this period, two variables were measured: the number of crossed cases and time of immobility.

RAM test The RAM test is performed to assess the memory of animals (Sandstrom and Hart 2005; Ciobica et al. 2010; Richter et al. 2013a, b). In our study, the device was star shaped with eight arms, 60 cm off the floor. It consisted of a central portion: four open arms and four closed with walls, each open arm facing a closed one. The assembly was made of wood. During the 5 min the test lasted, we tracked the mouse pathway in this device.

Preparation of brain homogenates

After 60 days of treatment, all animals were sacrificed by decapitation following deep ether anesthesia. The brains were collected and rinsed in phosphate-buffered saline, then homogenized in Tris NaCl buffer (50 mM tris, 15 mM NaCl, pH 7.4) using homogenizer Ultra-Torax and centrifuged at 7000 rpm at 4 °C for 15 min. The resulting supernatants were frozen at − 20 °C until further use.

Estimation of oxidative stress parameters

Determination of proteins Brain protein contents were determined as per Lowry et al. (1951), and bovine serum albumin was used as a standard.

Determination of lipid peroxidation levels Lipid peroxidation in brain tissue was determined by measuring thiobarbituric acid-reactive substances (TBARS) as per Draper and Hadley (1990).

Determination of PCO Tissue protein carbonyl (PCO) was measured by reacting 2,4-dinitrophénylhdrazine as per Levine et al. (1990). The absorbance was read at 370 nm and the results were expressed in nanomoles per milligram of protein.

Determination of AOPP levels Advanced oxidation protein product (AOPP) was determined as per Witko et al. (1992). The absorbance was measured at 340 nm using the molar extinction coefficient of 261 cm^{-1} $mmol^{-1}$ and the results were expressed in micromoles per milligram of protein.

Estimation of GPx activity The enzymatic activity of glutathione peroxidase (Gpx) was measured as per Flohé and Gunzler (1984).

Estimation of SOD activity The method used to assess the SOD activity in the mouse brain was described in Beauchamp and Fridovich (1971). This technique measures the ability of SOD to inhibit the photoreduction of nitroblue tetrazolium (NBT) into blue formazan. The activity was expressed in units per milligram of protein; one unit is the amount inhibiting the photoreduction of NBT by 50%.

Determination of AChE activity In the brain, acetylcholinesterase (AChE) activity was determined as per Ellman et al. (1961), using acetylthiocholine iodide as a substrate. The degradation of acetylthiocholine was measured at 412 nm. The result was expressed in micromoles of substrate hydrolyzed per mine per milligram of protein.

Brain histological studies

The sections of the brain were fixed, embedded in paraffin, and then 0.5-μm slices were stained with heymatoxylin-eosin. After drying, they were prepared and observed under the optical microscope.

Statistical analysis

All assays were performed in triplicate. Results are presented as mean ± standard error of the mean (SEM). Comparisons between groups ($n = 8$) were carried out by Student t test. Statistical significance was set at $p < 0.05$.

Results

Screening in vitro and choice of the best extract

Amounts of polyphenols and flavonoids The results presented in Table 1 show that all extracts contained abundant quantities of polyphenols and flavonoids; however, ethanolic extract (70% at 80 °C) contained the highest levels (100.46 mg GAE/g of extract and 130 mg QE/g of extract).

Evaluation of the antioxidant activity of *Curcuma longa* extracts using the DPPH test and TAA The DPPH test, presented in Table 2, revealed that ethanolic extract (70% at 80 °C) showed the highest antioxidant activity expressed by the lowest IC_{50} (53 μg/ml), compared to those of methanol 96% ($IC_{50} = 140$ μg/ml), ethanol 96% ($IC_{50} = 64$ μg/g), and ethanol 70% at 37 °C ($IC_{50} = 76$ μg/ml).

The same results were produced by the TAA test: ethanolic extract (70% at 80 °C) exhibited a higher TAA compared to other extracts (466.67 mg/g (Table 2)).

In vivo antioxidant effects of *Curcuma longa*

Body weight

Our results showed that the group of mice injected with $CeCl_3$ exhibited a significant decline in body weight as compared to the control group (Fig. 1). Coadministration of $CeCl_3$ and ECL alleviated the loss of body weight caused by cerium (Fig. 2).

Effects of *Curcuma longa* on behavioral and memory performance of $CeCl_3$-treated mice in the OF test

In the OF test (Fig. 3), the treatment had a significant effect on the total distance crossed by mice. A highly significant increase in the number of cases crossed in the $CeCl_3$-treated group and this number did not change from day 1 to day 15. The number of cases crossed in the $CeCl_3$+Curcuma.L-treated group was increased slightly in day 1 compared to the control group. However, the number of cases traveled decreased significantly from day 1 to day 15 of test with significant increase in immobility time (Table 3).

Radial 8-arm maze test

The results in Table 4 show that the $CeCl_3$-treated mice spent more time in closed arms than the control animals. The percentage of entries in closed arms, from day 1 to day 15 of the test, was higher compared to controls (Fig. 4). However, after ECL supplementation, these gravimetric disturbances were significantly attenuated. ECL treatment significantly increased the number of entries in open arms, which were gradually enhanced in the $CeCl_3$+Curcuma.L group.

Effect of *Curcuma longa* supplementation on oxidation biomarkers in the brain of $CeCl_3$ mice

The injection of $CeCl_3$ caused important protein oxidation associated with an elevation of PCO and AOPP (+ 336 and + 67%, respectively) compared to the control group. ECL supplementation significantly attenuated these effects in $CeCl_3$+Curcuma.L (Table 5).

Lipid peroxidation level (TBARS) increased in the brain tissue of the $CeCl_3$-treated group (+ 196% compared to control mice). On the contrary, in the $CeCl_3$+Curcuma.L-treated group, TBARS were reduced by 65% compared to those in the $CeCl_3$-treated group (Table 5).

No statistical changes were observed in the levels of PCO, AOPP, and TBARS between the control group and the group treated with ECL only ($p > 0.05$) (Table 5).

Table 2 Total phenolic and flavonoid contents and IC_{50} values of DPPH free radical scavenging and measure of total antioxidant activity. BHT and vitamin C were used as standards

	Total polyphenol	Total flavonoid	IC_{50} DPPH	AAT
Methanol	175.57 ± 3 mg/g	57.45 ± 0.5 mg/g	140 μg/ml	340 ± 34 mg/g
Ethanol 96% at 37 °C	66 ± 1.56 mg/g	51.92 ± 1.2 mg/g	64 μg/g	233.33 ± 57.7 mg/g
Ethanol 70%	106.23 ± 0.6 mg/g	59.94 ± 0.34 mg/g	76 μg/ml	173.33 ± 11.54 mg/g
Ethanol 70% at 80 °C	130.48 ± 2.1 mg/g	100.46 ± 1.5 mg/g	53 μg/ml	466.67 ± 115.47 mg/g
BHT	–	–	32.17 μg/ml	–

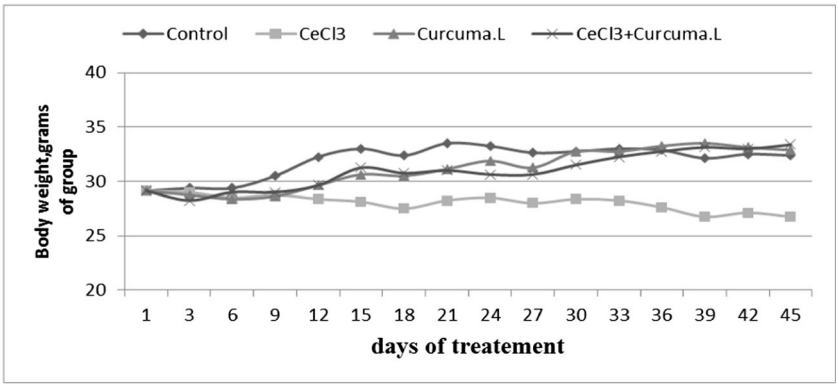

Fig. 1 Changes of body weight during the 45 days of the experimental period in mice treated with cerium chloride ($CeCl_3$) and cotreated with *Curcuma longa* ($CeCl_3$+ Curcuma.L)

Estimation of antioxidative enzyme activities

Our data (Fig. 5) showed that the administration of $CeCl_3$ caused a significant decrease in SOD (− 74%) and GSH-Px (− 69%) activity compared to the control group. However, the administration of ECL improved brain antioxidant activity compared to the $CeCl_3$-treated group. There was no difference in this activity between the mice treated only with *Curcuma longa* and the control group.

Effect of *Curcuma longa* on AChE activity

As shown in Fig. 6, AChE activity was significantly increased ($p < 0.001$) in the $CeCl_3$-treated group compared to the controls. However, the $CeCl_3$+Curcuma.L group had similar AChE activity as the controls.

Histological study of brain tissue

Figure 7 illustrates the histopathological alterations in the cortex of the treated groups compared to controls. Control mice showed normal histology (Fig. 7a). In the $CeCl_3$-treated mice, we observed retracted neurons with condensed chromatin undergoing necrosis or apoptosis and vacuolated spaces (Fig. 7b). In contrast, ECL cotreatment significantly prevented this damage and showed near normal brain tissue, similar to that of the controls (Fig. 7c). There were no histological alterations in the cerebral cortex of the ECL-treated group compared to controls (Fig. 7d).

Discussion

It is well known that the brain tissue is highly sensitive to oxidative damage caused by chemicals, including xenobiotics. Among these xenobiotics, cerium chloride has been shown to cause acute neurotoxicity in mice (Zhao et al. 2011). The aim of this work was to evaluate the potential protective effect of *Curcuma longa* against $CeCl_3$-induced neurotoxicity.

The study of four extracts of *Curcuma longa* showed that the plant is rich in polyphenols and flavonoids. These secondary metabolites have important antioxidant activity manifested by very low IC_{50} in the DPPH test and TAA. This corroborated with findings by Abbasi et al. (2012) demonstrating that *Curcuma longa* has powerful antioxidant and anti-inflammatory activities in various extracts of rhizome parts. The ethanolic extract (70% at 80 °C) was found to have the highest antioxidant activity in our study. The same result was obtained by Boullay et al. (2013). They showed that

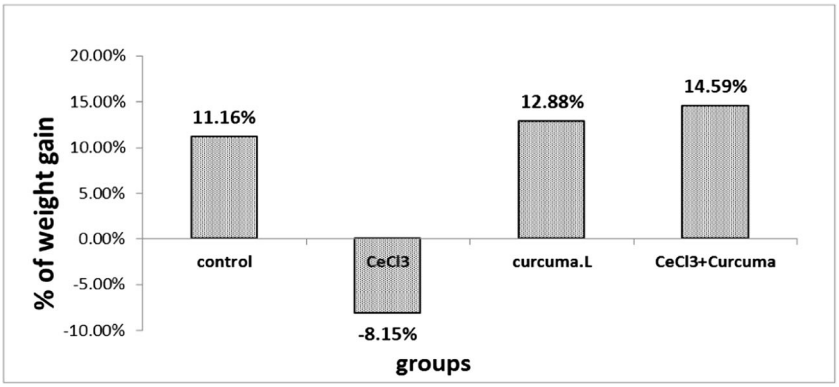

Fig. 2 Changes of body weight during the 45 days of the experimental period in mice treated with cerium chloride ($CeCl_3$) and cotreated with *Curcuma longa* ($CeCl_3$+ Curcuma.L)

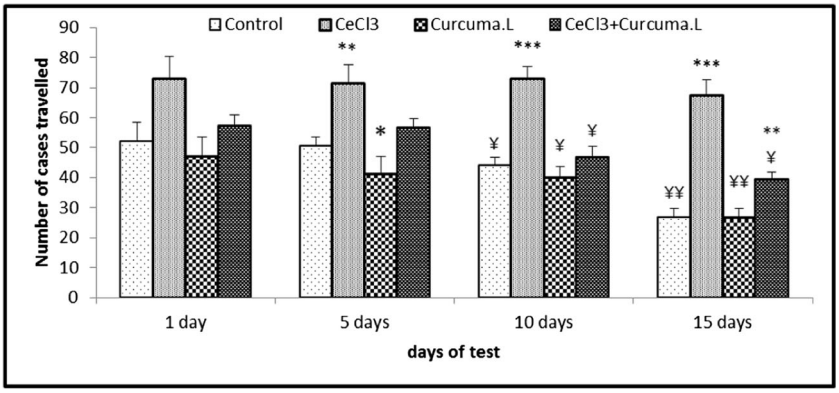

Fig. 3 Effects of chronic cerium and *Curcuma longa* administration. The effect on total distance traveled (case/5 min) by mice as determined in the open field test during 5 min. The values are mean ± SD of 8 animals. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different in comparison to control. ¥$p < 0.05$, ¥¥$p < 0.01$ significantly different in comparison to control in day 1 or day 5 of treatment

optimization of curcumin extraction (functional molecule of *Curcuma longa*) was achieved in the same conditions as ours.

The intraperitoneal injection of cerium chloride ($CeCl_3$) at 20 mg/kg BW decelerated body growth. This result was in accordance with previous studies (Cheng et al. 2011; Ze et al. 2011) reporting that weight gain was significantly decreased by $CeCl_3$ treatment at a dose of 20 mg/kg. The weight loss could be attributed to several causes, such as reduction of daily food consumption, inadequate absorption of nutrients, and inhibition of protein synthesis.

In our case, a daily administration of ECL to the $CeCl_3$-treated group stopped the weight loss. In fact, we can deduce that curcumin has an efficient role in the fight of body weight decline. Khan et al. (2012) observed that *Curcuma longa* could be used as a natural growth promoter in diet, due to its wide margin of safety and pharmacological properties. Moreover, Lee et al. (2010) reported that diet supplemented with *Curcuma longa* improved weight gain.

Brain biochemical alteration and neurobehavioral decline could result from neurotoxicity caused by environmental pollutants. Chronic exposure to cerium increases cerium concentration in the brain compared to normal. It has been observed that a high cerium level in the brain is associated with decline in visual memory (Zhao et al. 2011). The results of our study indicate that chronic administration of cerium chloride results in progressive deterioration of spatial memory in the open field and RAM tests. We noticed an increase in the number of cases crossed, accompanied by a decrease of immobility time in the OF and an impairment in learning ability during sessions of RAM in the $CeCl_3$ group. Experimentally, it has been shown that administration of cerium chloride causes learning deficits in the maze-Y test (Zhao et al. 2011) which is consistent with our results. However, adding ECL improved the memory of mice, which enhanced their performances; thus, the ECL significantly reduced mobility in the OF test and increased the number of entries in open arms in the RAM test. These results showed that the ECL could prevent cognitive deficits, suggesting a potential neuroprotective role against cerium neurotoxicity.

In the CNS, the cholinergic system plays an important role in learning and memory. Reduced function in this system is associated with memory loss and disorientation, such as those seen in Alzheimer's disease (Gibson and Duffy 1981). Experimentally, cerium chloride has been shown to increase acetyl-cholinesterase in the mouse brain (Zhao et al. 2011). This has been attributed to the accumulation of cerium in the brain. Our results indicated a significant increase in AChE activity in $CeCl_3$-treated mice. This may cause cholinergic transmission impairment, which is correlated with behavior abnormalities in mice. Further, increase in AChE activity contributes to decline in spatial recognition memory.

Moreover, our results revealed that ECL administration corrected memory impairment in $CeCl_3$-treated mice. This is related to the inhibition of AChE activity that improved

Table 3 Total immobility time (seconds) as determined in the open field test in 5 min.. The values are mean ± S.D. for 8 rats in each group

	Control	$CeCl_3$	Curcuma.L	$CeCl_3$ + Curcuma.L
Day 1	0.72 ± 0.46	0.88 ± 0.09	1.42 ± 0.43	1.08 ± 0.12
Day 5	1.79 ± 0.73	1.18 ± 0.92	1.52 ± 0.36	1.76 ± 0.41
Day 10	2.61 ± 0.58	0.85 ± 0.51**	2.63 ± 0.53#	3.47 ± 0.46##
Day 15	3.78 ± 0.27	0.64 ± 0.31***	3.65 ± 0.23##	3.55 ± 0.36##

The values are mean ± S.D. for 10 mice in each group

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$ significantly different as compared to the control

$\#p < 0.05$, $\#\#p < 0.01$ significantly different as compared to the $CeCl_3$-treated group

Table 4 Effect of *Curcuma longa* supplementation in mice treated or not treated with cerium chloride in the radial 8-arm maze (RAM) test in 5 min

		Control	$CeCl_3$	Curcuma.L	$CeCl_3$+Curcuma.L
Number of entries in open arms	J1	2.68 ± 0.56	1.79 ± 64	2.82 ± 0.48*	2.15 ± 0.79
	J5	2.89 ± 1.2	1.63 ± 0.48**	2.81 ± 0.84*#	2.6 ± 0.54
	J10	3.57 ± 0.35	0.52±0.41***	3.065 ± 0.38##	3.7 ± 0.41##
	J15	4.03 ± 0.33	0.29 ± 0.28***	3.41 ± 0.39##	3.7 ± 0.41##
Number of entries in close arms	J1	1.91 ± 0.06	2.68 ± 0.68	2.08 ± 0.04	1.7 ± 0.41
	J5	1.51 ± 0.21	2.9 ± 0.48	2.01 ± 0.84	1.82 ± 0.54
	J10	0.44 ± 0.04	3.82 ± 0.41**	1.12 ± 0.45##	0.77 ± 0.63#
	J15	0.14 ± 0.02	4.23 ± 0.2***	0.56 ± 0.03##	0.46 ± 0.04##
Time spent in the open arms	J1	3.25 ± 0.5	4.25 ± 1.5	5.25 ± 1.71	4.5 ± 1.3
	J5	6.5 ± 2.08	3.75 ± 0.9	6.75 ± 1.7	6.5 ± 0.58
	J10	9 ± 1.4	1.75 ± 1.25**	10.25 ± 2.21##	8.75 ± 1.26##
	J15	12.5 ± 2.89	4.26 ± 0.82***	9.5 ± 3.7##	12 ± 2.9##
Time spent in the close arms	J1	5 ± 1.83	6 ± 1.63	9 ± 2.2	8 ± 2.58
	J5	3.25 ± 0.9	7.5 ± 1	7 ± 1.4	5.5 ± 2.65
	J10	2.75 ± 1.71	9 ± 2.16**	4.25 ± 1.71#	3.75 ± 2.3##
	J15	1.25 ± 0.96	11.5 ± 1.73***	4.25 ± 0.08##	1 ± 0.3##

The values are mean ± S.D. for 10 mice in each group

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significantly different as compared to the control

#$p < 0.05$, ##$p < 0.01$ significantly different as compared to the $CeCl_3$-treated group

cholinergic neural transmission. In vivo and in vitro models have demonstrated the inhibitory effect of *Curcuma longa* on the activities of cholinesterase (Jaques et al. 2011). These authors demonstrated that curcumin was effective in the prevention of AChE activity increase in the striatum and cerebral cortex. Anil et al. (2009) demonstrated that curcumin crosses the blood-brain barrier; this provides additional evidence for its neuroprotective effect against toxicological damage.

In biological systems, oxidative stress is the consequence of an imbalance between the production of free radicals and the capability of the antioxidant defense system. ROS production can be amplified by different physiological mechanisms (inflammation) or environmental factors (medication, chemicals, heavy metals, pesticides, alcohol, and others). ROS include superoxide anions ($O_2^{\cdot}$), H_2O_2, and hydroxyl radical ($OH^{\cdot}$) (Foyer and Noctor 2011) which induce oxidation of proteins, lipids, and nucleic acids (Nielsen et al. 2003). Our results suggested that $CeCl_3$ treatment induced a remarkable TBARS level increase in mouse brain. Lipid peroxidation (LPO) is a major effect of free radicals; cell membranes are rich in unsaturated fatty acids and are, therefore, very sensitive to oxidative stress. Thus, aldehydes produced after membrane oxidation participate in chain reactions that increase damage to biomolecules (Cui et al. 2004). It has been found that $CeCl_3$ induces LPO and alters the physiological and biochemical characteristics of biological systems (Zhao et al. 2010; Fei et al. 2011). Cerium chloride makes an important production of free radicals $OH^{\cdot}$ and $O^{\cdot}_2$ which are very reactive and able to oxidize unsaturated lipids of the cell membranes. We also observed a significant increase in PCO and AOPP levels in $CeCl_3$-treated brains, which confirms that $CeCl_3$ induced the formation of free radicals and oxidation

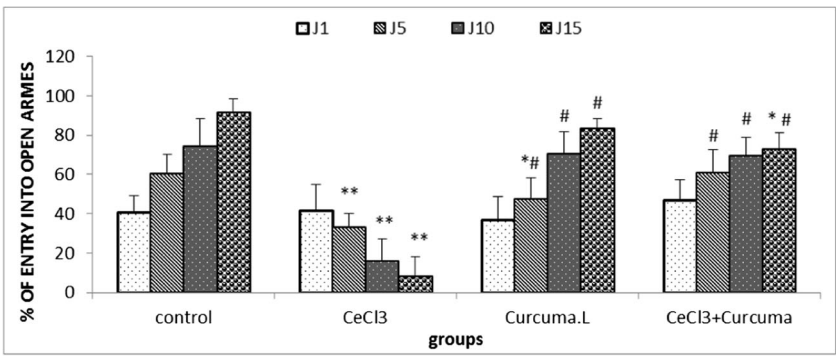

Fig. 4 Percentage of entry in the open arms in the mice treated with $CeCl_3$ and $CeCl_3$+Curcuma.L during the 15 days of the test. The values are mean ± SD of 8 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significantly different in comparison to control. #$p < 0.05$ different in comparison to $CeCl_3$-treated mice

Table 5 Effects of *Curcuma longa* administration on the lipid peroxidation (TBARS levels), carbonyl protein (PCO), and advanced oxidation protein product (AOPP) in brain homogenate, in all experimental groups

	Control	$CeCl_3$	Curcuma.L	$CeCl_3$+Curcuma.L
TBARS (nmol of TBARS/mg protein)	2.45 ± 0.19	7.25 ± 1.19**	2.65 ± 0.23#	2.75 ± 0.45#
PCO (nmol/mg protein	0.53 ± 0.09	2.31 ± 0.96**	0.82 ± 0.22*#	0.66 ± 0.23#
AOPP (μmol/mg protein)	0.024 ± 0.002	0.04 ± 0.01**	0.03 ± 0.008	0.02 ± 0.004#

The values are mean ± S.D. for 10 mice in each group

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significantly different as compared to the control

#$p < 0.05$, ##$p < 0.01$ significantly different as compared to the $CeCl_3$-treated group

of protein in the brain. A significant reduction in brain TBARS, PCO, and AOPP levels was observed in the $CeCl_3$+Curcuma.L group compared to the $CeCl_3$-treated group. These results confirmed the protective effect and antioxidant capacity of *Curcuma longa* against oxidative damage. The protective effect of ECL on membrane lipids and proteins was mediated by ROS neutralization by the phenolic and flavonoid compounds present in the plant.

According to Liu et al. (2010) and Uzun et al. (2010), the enzymatic antioxidants SOD and GPx play a very important role during the scavenging process of reactive oxygen species. Our data showed that SOD and GPx activities were highly

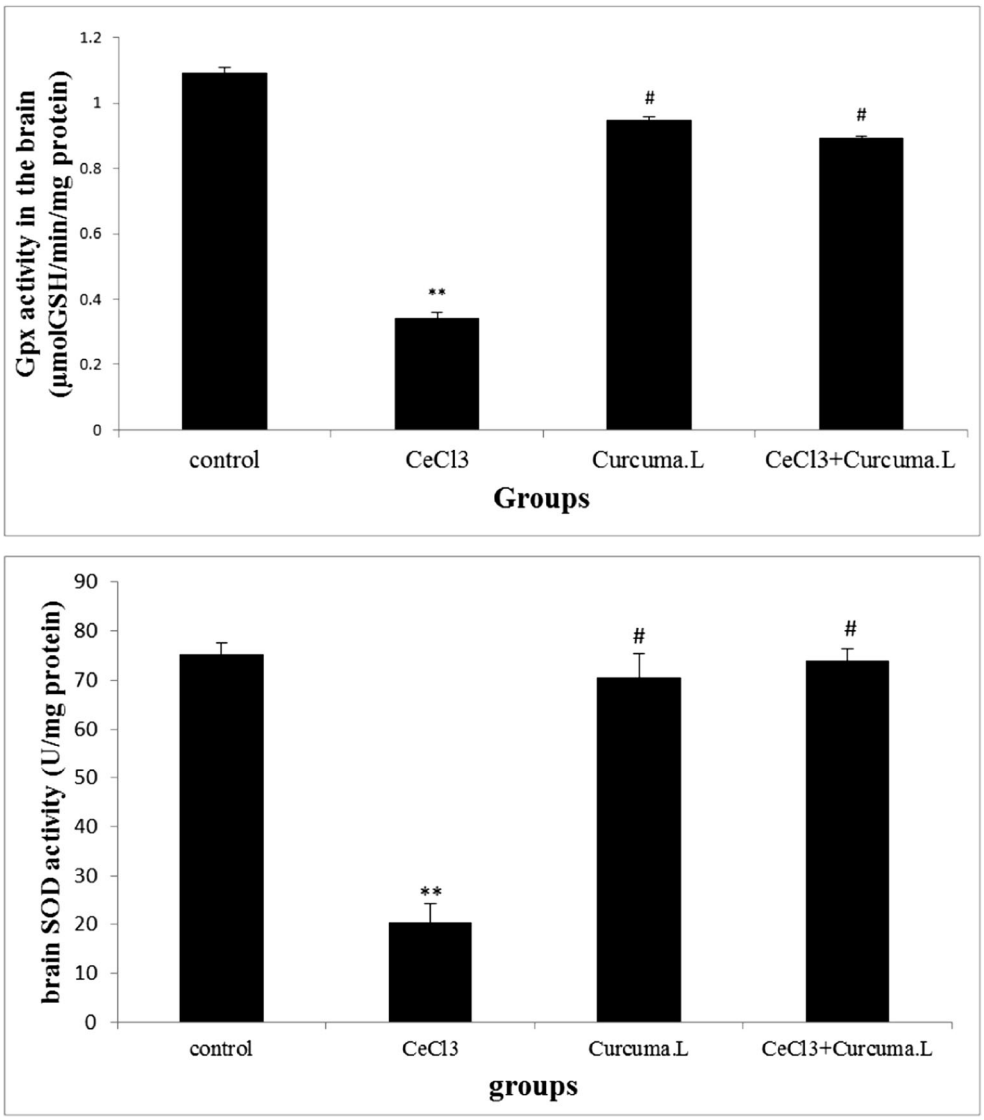

Fig. 5 Antioxidant activities of SOD and GPx in the brains of $CeCl_3$, $CeCl_3$+Curcuma.L, and Curcuma.L during the 60 days. Data are expressed as mean ± S.D. for 8 animals in each group. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ significantly different as compared to the control group. #$p \leq 0.05$, ##$p \leq 0.01$ significantly different as compared to the $CeCl_3$-treated group

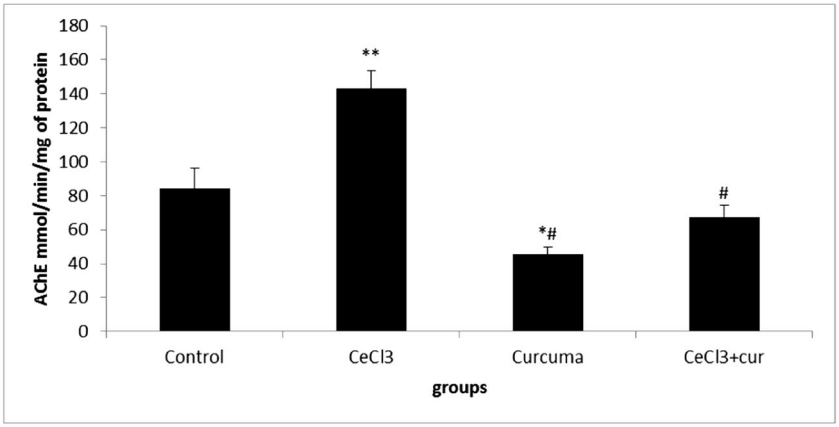

Fig. 6 Effects of *Curcuma longa* extract administration on acetylcholinesterase in brain homogenate, in all experimental groups. Data are expressed as mean ± S.D. for 8 mice in each group. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ significantly different as compared to the control group. $\#p \leq 0.05$, $\#\#p \leq 0.01$ significantly different as compared to the $CeCl_3$-treated group

reduced in the $CeCl_3$-treated group. Indeed, the decrease in SOD and Gpx activity indicated a physiological and molecular response to the disruption of redox equilibrium. Numerous studies have confirmed our results (Xiao et al. 2008; Peil et al. 2011; Zhao et al. 2011). These authors also found that $CeCl_3$ decreases the antioxidant activity of SOD and GPx in the brain.

We found that *Curcuma longa* treatment was able to restore the activity of these antioxidant enzymes (GPx and SOD) in cerium chloride-treated mice. This restoration was due to the richness of *Curcuma longa* in polyphenol, flavonoids, antocianne, and polysaccharide (Julie and Jurenka 2009). In this context, Koiram et al. (2007) reported that curcumin increases the levels of SOD and catalase in irradiated mice.

Brain tissue study is primordial to understand the impact of $CeCl_3$ on neurobehavioral and memory activity. Our results showed that $CeCl_3$ caused pyknotic nucleus, vacuolated cytoplasm, and cellular depletions in treated mouse brain. Interestingly, chronic treatment with ECL clearly reduced these damages and ameliorated the

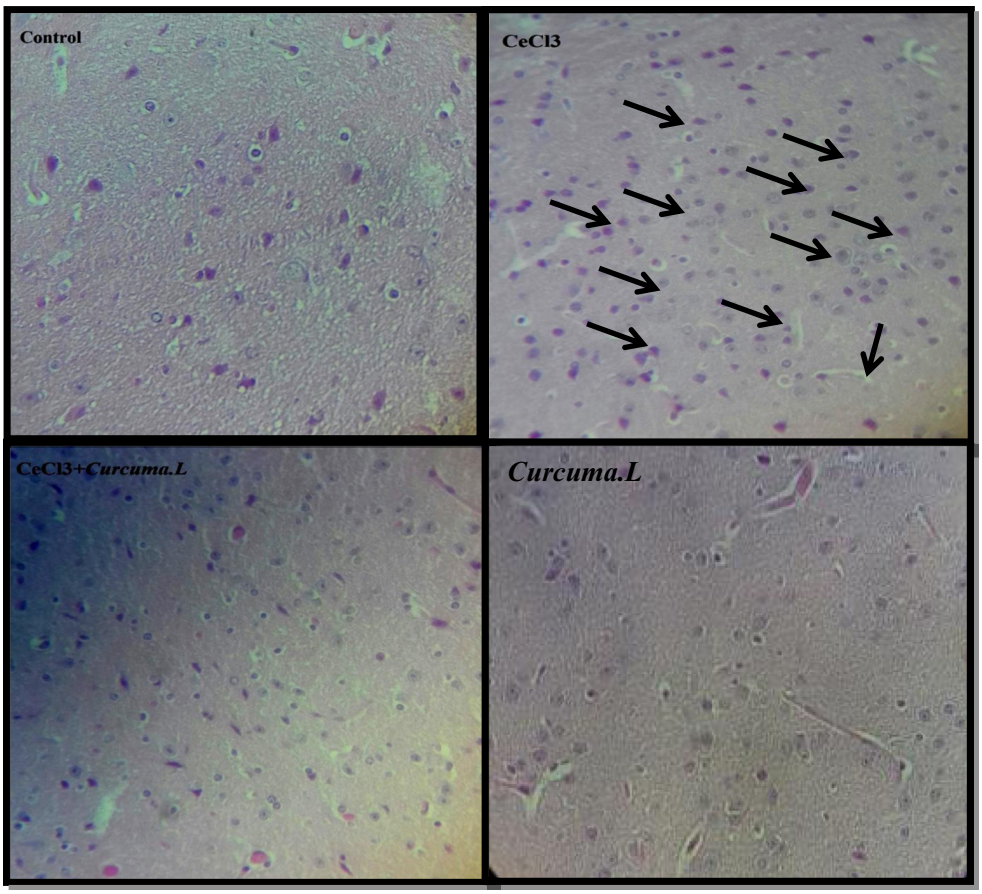

Fig. 7 Brain paraffin section photograph(s) of adult mouse controls and experimental groups, controls, $CeCl_3$, $CeCl_3$+ Curcuma.L, and Curcuma.L showing histopathological changes (arrows)

histological structure. This proved the protective effect of *Curcuma longa* against $CeCl_3$ exposure and confirmed our results suggesting that *Curcuma longa* can prevent oxidative stress damage in cerebral tissues.

Conclusion

Overall, in this work, we have demonstrated that chronic cerium treatment caused a disruption in redox hemostasis, cholinergic system, and a histopathological alteration in mouse brain. However, the ethanolic extract of *Curcuma longa* showed a protective effect against cerium toxicity and was able to prevent the Ce brain damage. Taken together, the findings demonstrated that extract of *Curcuma longa* (ECL) treatment significantly ameliorated the cognitive impairment and attenuated oxidative stress markers in Ce-exposed mice.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

References

Abbasi MA, Ilyas M, Sonia A, Shahwar D, Raza MA, Khan KM, Ashraf M, Afzal I, Ambreen N (2012) Curcumin and its derivatives: moderate inhibitors of acetylcholinesterase, butyrylcholinesterase and trypsin. Sci Iran 19:1580–1583

Allagui MS, Feriani A, Saoudi M, Badraoui R, Bouoni Z, Nciri R, Murat JC, Elfeki A (2014) Effects of melatonin on aluminium-induced neurobehavioral and neurochemical changes in aging rats. Food Chem Toxicol 70:84–93

Anil SS, Anil L, Deen J (2009) Effect of lameness on sow longevity. J Am Vet Med Assoc 235(6):734–738

Barros L, Baptista P, Ferreira IC (2007) Effect of Lactarius piperatus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food Chem Toxicol 45:1731–1737

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Beltifa A, Borgi MA, Ferieni A, Elfekih A, Mansour HB, Allagui MS (2017) In vivo protective role against water contamination with cerium via chronic administration of omega 3. Environ Sci Pollut Res Int 24(1):146–151

Boullay V, Plaza M, Capelle L, Chaby L (2013) Identification of emotions in patients with low-grade gliomas versus cerebrovascular accidents. Rev Neurol (Paris) 169(3):249–257

Cheng J, Li N, Cheng Z, Hua R, Cai J, Si W, Hong F (2011) Splenocyte apoptotic pathway in mice following oral exposure to cerium trichloride. Chemosphere 83(4):612–617

Ciobica A, Hritcu L, Padurariu M, Dobrin R, Bild V (2010) Effects of serotonin depletion on behavior and neuronal oxidative stress status in rat: relevance for anxiety and affective disorders. Adv Med Sci 55:289–296

Cui K, Luo X, Xu K, Murthy MV (2004) Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. Prog Neuro-Psychopharmacol Biol Psychiatry 28:771–799

Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88IN191–88I9095

Fei M, Li N, Ze Y, Liu J, Gong X, Duan Y, Zhao X, Wang H, Hong F (2011) Oxidative stress in the liver of mice caused by intraperitoneal injection with lanthanoides. Biol Trace Elem Res 139(1):72–80

Flohé L, Günzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–21

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, Sharma RA, Steward WP, Gescher AJ (2005) Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. Cancer Epidemiol Biomark Prev 14:120–125

Gibson GE, Duffy TE (1981) Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. J Neurochem 36:28–33

İlhami G (2012) Antioxidant activity of food constituents: an overview. Arch Toxicol 86:345–391

Jaques JA, Rezer JF, Gonçalves JF, Spanevello RM, Gutierres JM, Pimentel VC, Thomé GR, Morsch VM, Schetinger MR, Leal DB (2011) The effect of curcumin in the ectonucleotidases and acetylcholinesterase activities in synaptosomes from the cerebral cortex of cigarette smoke-exposed rats. Cell Biochem Funct 29(8):703–707

Julie S, Jurenka MT (2009) Anti-inflammatory properties of curcumin, a major constituent. Altern Med Rev 14:141–153

Khan RU, Naz S, Javdani M, Nikousefat Z, Selvaggi M, Tufarelli V, Laudadio V (2012) The use of turmeric (Curcuma longa) in poultry feed. J World Poul Sci 68(01):97–103

Kilbourn TB (2003) The role of the lanthanides in applied catalysis. J Less-Common Met 126:101–106

Kohli K, Ali J, Ansari MJ, Raheman Z (2005) Curcumin: a natural antiinflammatory agent. Indian J Pharmacol 37(3):141

Koiram PR, Veerapur VP, Kunwar A, Mishra B, Barik A, Priyadarsini IK, Mazhuvancherry UK (2007) Effect of curcumin and curcumin copper complex (1:1) on radiation-induced changes of anti-oxidant enzymes levels in the livers of Swiss albino mice. J Radiat Res 48(3): 241–245

Lee SH, Lillehoj HS, Jang SI, Kim DK, Ionescu C, Bravo D (2010) Effect of dietary curcuma, capsicum, and lentinus, on enhancing local immunity against Eimeria acervulina infection. J Poul Sci 47:89–95

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186: 464–478

Li H, Wong C, Cheng K, Chen F (2008) Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT–Food Sci Technol 41:385–390

Liu AR, Chen SC, Lin XM, Wu SY, Xu T, Cai FM, Raesh J (2010) Endophytic Pestalotiopsis species spp. associated with plants of Palmae, Rhizophoraceae, Planchonellae and Podocarpaceae in Hainan, China. Afr J Microbiol Res 4:2661–2669

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Nielsen R, Tarpy DR, Reeve HK (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. Mol Ecol 12:3157–3164

Nwozo S, Adaramoye O, Ajaiyeoba E (2009) Oral administration of extract from Curcuma longa lowers blood glucose and attenuates alloxan-induced hyperlipidemia in diabetic rabbits. Pak J Nutr 8: 625–628

Osawa T, Sugiyama Y, Inayoshi M, Kawakishi S (1995) Antioxidative activity of tetrahydrocurcuminoids. Biosci Biotechnol Biochem 59: 1609–1612

Peil OE, Georges A, Lechermann F (2011) Strong correlations enhanced by charge ordering in highly doped cobaltates. Phys Rev Lett 107(23):236404

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin JC, Bailleul F, Trotin F (2000) Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J Ethnopharmacol 72:35–42

Reinhardt K, Winkler H (2002) Cerium mischmetal, cerium alloys, and cerium compounds. UEIC 7:285–300

Richter SH, Zeuch B, Lankisch K, Gass P, Durstewitz D, Vollmayr B (2013a) Where have I been? Where should I go? Spatial working memory on a radial arm maze in a rat model of depression. PLoS One 8(4):e62458

Richter T, Buhse S, Kupfer R, Gerlach A, Mühlhauser I, Lenz M (2013b) Development of a decision aid on post mortem organ donation—somewhere between evidence uncertainty, fears, and ethical and moral values. Z Evid Fortbild Qual Gesundhwes 107(9–10):622–631

Sandstrom NJ, Hart SR (2005) Isolation stress during the third postnatal week alters radial arm maze performance and corticosterone levels in adulthood. Behav Brain Res 156:289–296

Sethi P, Jyoti A, Singh R, Hussain E, Sharma D (2008) Aluminium-induced electrophysiological, biochemical and cognitive modifications in the hippocampus of aging rats. Neurotoxicology 29(6): 1069–1079

Sethi P, Jyoti A, Hussain E, Sharma D (2009) Curcumin attenuates aluminium-induced functional neurotoxicity in rats. Pharmacol Biochem Behav 93(1):31–39

Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM, Pirmohamed M (2004) Phase I clinical trial of oral curcumin. Clin Cancer Res 10(20):6847–6854

Tønnesen HH, Karlsen J (1986) Studies on curcumin and curcuminoids VII. Chromatographic separation and quantitative analysis of curcumin and related compounds. Z Lebensm Unters Forsch 182(3):215–218

Topal M, Gocer H, Tpal F, Kalin P, K se PL, İlhami G, Çakmak CK, Küçük M, Durmaz L, Gören CA, Alwasel SH (2016) Antioxidant, antiradical, and anticholinergic properties of cynarin purified from the Illyrian thistle (Onopordum illyricum L.). J Enzyme Inhib Med Chem 31(2):266–275

Tuba Ak İlhami G (2008) Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 174:27–37

Uzun A, Gulsen O, Kafa G, Seday U (2010) Peroxidase genebased estimation of genetic relationships and population structure among Citrus spp. and their relatives. Field performance and molecular diversification of lemon selections. Sci Hortic 120:473–478

Vinod KG, Singh AK, Barkha G (2006) A cerium (III) selective polyvinylchloride membrane sensor based on a Schiff base complex of N, N-bis [2-(salicylideneamino) ethyl] ethane-1, 2-diamine. Anal Chim Acta 575:198–204

Witko V, Nguyen AT, Descamps-Latscha B (1992) Microtiter plate assay for phagocyte-derived taurine chloramines. J Clin Lab Anal 6:47–53

Xiao K, Dalgarno KW, Wood DJ, Goodridge RD, Ohtsuki C (2008) Indirect selective laser sintering of apatite-wollostonite glass-ceramic. Proc Inst Mech Eng H 222(7):1107–1114

Záveská E, Fér T, Šída O, Marhold K, Leong-Škorničková J (2016) Hybridization among distantly related species: examples from the polyploid genus Curcuma(Zingiberaceae). Mol Phylogenet Evol 100:303–321

Ze Y, Cheng J, Cai J, Cheng Z, Hu R, Hong F (2011) The impairment of liver DNA conformation and liver apoptosis of mice caused by CeCl3. Biol Trace Elem Res 143(1):437–445

Zhao H, Cheng Z, Hu R, Jie C, Hong M, Zhou M, Gong X, Wang L, Hong F (2010) Oxidative injury in the brain of mice caused by Lanthanid. Biol Trace Elem Res 142:174–189

Zhao H, Cheng Z, Cheng J, Hu R, Che Y, Cui Y, Wang L, Hong F (2011) The toxicological effects in brain of mice following exposure to cerium chloride. Biol Trace Elem Res 144(1–3):872–884

Zhao H, Jie C, Jingwei C, Zhe C, Yaling C, Guodong G, Renping H, Xiaolan G, Ling W, Fashui H (2012) Liver injury and its molecular mechanisms in mice caused by exposure to cerium chloride. Arch Environ Contam Toxicol 62:154–164