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Curcuminoids, Curcumin, and Demethoxycurcumin Reduce Lead-Induced Memory Deficits in Male Wistar Rats

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This study investigated the neuroprotective effects of the curcuminoids against lead-induced neurotoxicity. The results show that lead significantly increases lipid peroxidation and reduces the viability of primary hippocampal neurons in culture. This lead-induced toxicity was significantly curtailed by the co-incubation of the neurons with the curcuminoids. In a whole animal experiment, rats were trained in a water maze and thereafter dosed with lead and/or curcumin (CURC), demethoxycurcumin (DMC), or bisdemethoxycurcumin (BDMC) for 5 days. Animals treated with curcumin and demethoxycurcumin but not bisdemethoxycurcumin had more glutathione and less oxidized proteins in the hippocampus than those treated with lead alone. These animals also had faster escape latencies when compared to the Pb-treated animals indicating that CURC- and DMC-treated animals retain the spatial reference memory. The findings of this study indicate that curcumin, a well-established dietary antioxidant, is capable of playing a major role against heavy metal-induced neurotoxicity and has neuroprotective properties.

KEYWORDS: Curcumin; antioxidant; lipid peroxidation; protein oxidation; glutathione; spatial reference memory; neuroprotection

INTRODUCTION

The polyphenolic flavonoid curcumin (CURC) found in turmeric is a yellow curry spice with a long history of use in traditional Indian diets and herbal medicine (*1*, *2*). Curcumin (diferuloyl methane) from *Curcuma longa* has many pharmacological activities including anti-inflammatory properties (*3*), powerful antioxidant activity (*4*), anti-protease activity (*5*), and cancer preventative properties (*6*). It has also been reported that curcumin is a more potent free radical scavenger than vitamin E (*7*). Studies have shown that curcumin is a powerful scavenger of the superoxide anion, the hydroxyl radical, and nitrogen dioxide (*8*) and that it also protects DNA against singlet-oxygen-induced strand breaks (*9*) and lipids from peroxidation (*10*). Oral administration of curcumin has been shown to be centrally neuroprotective (*11*). In addition to curcumin, the *C. longa* rhizome also contains structurally related compounds demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC).

The neuroprotective effects of curcumin are not fully understood. Apart from the antioxidant and free radical scavenging properties of curcumin, another possibility is that it could act as a metal chelator. Metals play an important role in neural toxicity. Research has shown the involvement of metals in neurodegenerative disorders such as Alzheimer's disease, prion disease, mitochondrial disorders, Wilson's disease, and Parkinson's disease (*12*). The heavy metal lead (Pb) is known to be toxic even at low concentrations and exerts extensive damage to the brain, causing severe learning and memory disabilities. Lead has been characterized by the U.S. surgeon general as "one of the greatest environmental threats in America". This was based on the observation that 1 in 20 children under the age of 6 with blood Pb levels greater than 10 μg/dL (*13*) exhibited deficits in cognitive function and behavior (*14*). This heavy metal has also been shown to induce oxidative damage (*15*, *16*). Recent studies show that Pb may be toxic through the disruption of the delicate prooxidant/antioxidant balance that exists within mammalian cells (*15*). In vivo studies suggest that Pb is capable of generating reactive oxygen species, thus altering the antioxidant defense systems in animals (*17*). In a previous study we demonstrated that curcumin reduces Pb- and cadmium-induced neurotoxicity in rat hippocampal neurons through its antioxidant and metal binding properties (*16*).

In the present study we examined the neurotoxic effects of Pb and its ability to affect spatial reference memory. We also examined the potential of the three major curcuminoids in *C. longa* to protect against heavy-metal-induced neurotoxicity.

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MATERIALS AND METHODS

Chemicals and Reagents. Curcumin (65% pure), lead acetate, butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane (99%), MTT (3,(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide), trypsin, trypsin inhibitor, trypan blue, cytosine-β-D-arabinofuranoside, 2,4-dinitrophenylhydrazine, 5,5′-dithiobis-nitrobenzoic acid (DTNB), and reduced glutathione (GSH) were purchased from Sigma Chemicals Corporation, St. Louis, MO. Trichloroacetic acid (TCA), ethanol, methanol, dichloromethane, and butanol were purchased from Saarchem, Johannesburg, South Africa. Eagle's Minimum Essential Medium (MEM), foetal calf serum (FCS), and sterile disposable cell scrapers were purchased from Highveld Biologicals, South Africa. Tissue culture flasks (25 cm^3) and Falcon tubes were purchased from Corning Costar (USA). Sodium benzylpenicillin (Novopen), Streptomycin sulfate (Novo-Strep) and Amphotericin B (Fungizone) were supplied by Novo Nordisk (Pty) Ltd., South Africa. Semipreparative aluminum thin layer chromatography (TLC) plates coated with silica gel 60, Type F254 (0.25 mm) were purchased from Merk, Darmstadt, Germany.

Isolation of the Curcuminoids from Curcumin (65% Pure). The curcuminoids were separated by employing preparative TLC. Curcumin (65% pure) was dissolved in dichloromethane to produce a 0.05 g/mL solution and was spotted onto the TLC plates. The plates were developed in a tank containing dichloromethane and methanol (97:3) as the eluent. Once developed, each band was cut out, the silica was scraped off the plates, and the curcuminoids were re-extracted from the silica using dichloromethane. The dichloromethane was evaporated in vacuo. The purity of the curcuminoids obtained was tested by thin layer chromatography, nuclear magnetic resonance (NMR) and mass spectrometry (MS). More details are available in the Supporting Information section.

Animal Care. Adult male and female rats of the Wistar strain, weighing between 250 and 300 g, were purchased from South African Institute for Medical Research (Johannesburg, South Africa). The animals were housed in a controlled environment with a 12 h light: dark cycle, and they were given access to food and water ad libitum. The Rhodes University animal ethics committee approved protocols for the experiments.

Cell Culture. Hippocampi were dissected out of 1 day old rat pups. The tissue was chopped into fine pieces and thereafter triturated several times using a fire-polished pipet to yield dissociated cells. The sample was then incubated for 10 min at 37 °C in 0.1% trypsin, centrifuged to collect the pellet, and resuspended in MEM. This stock was diluted to obtain a density of 1×10^6 viable cells per 1 mL of MEM. A hemeocytometer was used to count viable neurons which exclude trypan blue. MEM was supplemented with FCS to produce a final concentration of 16% (v/v). This was also supplemented with Penicillin (100 U/mL), Streptomycin (100 μg/ mL), Amphotericin B (2.5 μg/mL), and cytosine (10 μM final concentration). Neurons were allowed to attach to the surface of the culture flask for 12−18 h, after which the MEM was discarded and replaced with 3 mL of fresh MEM. This MEM was supplemented with 10% FCS and 5 μM cytosine. Cell culture flasks were kept in an incubator set at 37 °C, the humidity was maintained between 75 and 80%, and the CO_2 was kept at approximately 5−7%. Cultures were bathed regularly with sterile phosphate buffered saline (PBS), pH 7.4. Six to eight day old cultures were used for the experiments. Before experimentation, each flask was washed twice with 3 mL of PBS. Thereafter 1 mL of fresh MEM was added. Cells were incubated for 3 h with lead acetate (10 μM) with and without varying concentrations of the curcuminoids (50, 100, and 150 μM). Controls were free of lead and curcuminoids. Following the 3 h incubation period, cell culture flasks from each group were used for the lipid peroxidation and cell survival assays.

Lipid Peroxidation Assay. A modified method of (*18*) was used to measure lipid peroxidation.

After the 3 h incubation period, cells were scraped off the surface of the culture flasks using a cell scraper and transferred to test tubes. After centrifuging for 10 min at 600*g*, the media was discarded and the cells were re-suspended in 1.1 mL of PBS. A 100 μL aliquot was removed for enumeration using a hemeocytometer. To the 1 mL of sample that remained were added 0.5 mL BHT (0.05% in ethanol) and 1 mL TCA (25%). These were boiled for 10 min to release protein bound malondialdehyde (MDA), cooled, and centrifuged at 2000*g*. Following centrifugation, 2 mL of the protein free supernatant was removed from each tube and 0.5 mL aliquots of 0.33% TBA were added to this fraction. All tubes were heated for 1 h at 95 °C in a water bath. After cooling, the TBA-MDA complexes were extracted with 2 mL of butanol. The absorbance was read at 532 nm, and MDA levels were determined from a standard curve generated from 1,1,3,3-tetramethoxypropane. Final results are represented as nmoles MDA/10^6 cells.

Assay for Cell Survival. Cell survival was evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay as described in ref *19* with minor modifications.

After the 3 h incubation period, the cells were gently washed thrice with 3 mL of PBS and 1 mL of fresh MEM added. The cells were loaded with 0.5 mL of MTT dye at a final concentration of 1 mg/mL. The cells were further incubated for 2 h after which the cells were scraped off the culture flasks using a cell scraper and transferred to a test tube. The culture flasks were rinsed with 1 mL of ice cold PBS and transferred to the test tubes. The test tubes were centrifuged for 15 min at 3000*g* after which the supernatant was carefully poured off and the resulting pellet dissolved in DMSO. The absorbance was read at 570 nm. The results were expressed as percentage viability/10^6 cells. The controls were taken as 100% viable.

Animal Treatment Protocol. For the purposes of intraperitoneal injections, the curcuminoids were solubilized in ethyl oleate and injected at a dose of 30 mg/kg/bd for 5 days, and lead acetate was dissolved in Milli-Q water and injected at a dose of 20 mg/kg/od for 5 days (*16*). The curcuminoids were injected every morning between 8:00 and 9:00 and every evening between 17:00 and 18:00. Pb was injected at 12:00. Five animals from each group were sacrificed on the morning of the sixth day by cervical dislocation, and their hippocampi were analyzed for the protein carbonyl and GSH content. These animals were referred to as the day = 1 animals. Animals from the behavioral studies group were sacrificed at the end of the study, (14 days later), and their hippocampi were also analyzed for the protein carbonyl and GSH content. These animals were referred to as the day = 14 animals. All brains were rapidly removed, dissected, frozen in liquid nitrogen, and stored at −70 °C until use.

Water Maze Task. The water maze task was carried out for 14 days after the treatment regime. The apparatus consisted of a circular water tank (150 cm in diameter and 40 cm high). A platform (12.5 cm in diameter and 31 cm high) invisible to the rats, was set inside the tank and filled with water maintained at approximately 23 °C at a height of 33 cm. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. Animals were trained for 4 days before being treated. For each training session, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. Training was conducted for 4 consecutive days, twice a day, with each session consisting of 4 trials. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall. The time taken for each rat to find the hidden platform from the starting point was measured by a person unaware of the experimental conditions.

Protein Carbonyl Measurement. The assay was conducted according to a modified method of (*20*).

Rat hippocampi homogenates (10% w/v in PBS) were diluted with buffer to 5 mg/mL. To this was added streptomycin sulfate (1% final concentration), and the mixture was gently stirred for 15 min and thereafter centrifuged at 11000*g* for 10 min at 4 °C. The supernatant was collected and diluted to 1 mg/mL. TCA was added at a final concentration of 10% to precipitate proteins. Samples were centrifuged at 11000*g*, and the pellet was recovered and diluted in buffer to yield a 1 mg/mL suspension. An aliquot of 0.5 mL of 2,4-dinitrophenylhydrazine (2 mM final concentration) was added to each tube, and the mixture was incubated at room temperature for 1 h with vortexing every 10−15 min. Proteins were precipitated with TCA and recovered after centrifuging for 10 min at 11000*g*. The pellet was washed twice with a 1 mL mixture of ethanol and ethyl acetate (1:1) and then dissolved

in 0.6 mL of 6 M guanidine. The absorbance was read at 370 nm, and the protein carbonyl content was calculated using a molar absorption coefficient of 22000 $M^{-1}cm^{-1}$. Results are expressed as nmol/mg protein. Protein estimation was performed using the method described by (*21*).

Glutathione Measurement. Glutathione levels were measured using a method previously described in ref *22*. Briefly, to 0.5 mL of rat hippocampi homogenates (5% w/v in PBS) were added 1.5 mL of 0.2 M Tris-HCl (pH 8.2), 0.1 mL of 0.01 M DTNB, and 7.9 mL of methanol, and the mixture was incubated for 30 min at room temperature. Thereafter the incubation mixture was centrifuged at 3000*g* for 15 min, and the supernatant read at 412 nm. The glutathione levels were determined from a standard curve generated from GSH and are expressed as nmol GSH/mg tissue.

Statistical Analysis. The GSH, protein carbonyl, lipid peroxidation, and cell viability assay results were analyzed using a one-way analysis of the variance (ANOVA) followed by the Student−Newman−Keuls Multiple Range Test. The level of significance was accepted at $p < 0.05$. The behavioral study results were analyzed using repeated measures ANOVA with group as a factor and latency measured over 14 days. Scheffes' test for multiple group comparison was used for post hoc analysis. The level of significance used was $\alpha = 0.05$.

RESULTS

Curcumin (65% pure) produced three distinct spots on the TLC plate with R_f values of 0.83, 0.61, and 0.5 belonging to CURC, DMC, and BDMC, respectively. The purified fractions of the curcuminoids show only one spot each with each fraction having the respective R_f value. ^{13}C NMR (DMSO-d_6, 100 MHz) and ESI-MD data of the curcuminoid fractions indicate the presence of 21, 20, and 19 carbons for CURC, DMC, and BDMC, respectively. CURC shows two shifts at δ 55.6 indicating the presence of two methoxy groups ,while DMC shows one shift, and none were observed for BDMC. CURC shows no contamination by the other curcuminoids and was thought to be more than 95% pure by both NMR and mass spectroscopy. The DMC obtained was pure by ^1H and ^{13}C NMR spectroscopy, but it was found to contain an insignificant amount of CURC (10% relative abundance to the DMC molecular ion). The NMR spectra of BDMC indicated a small amount of contamination by DMC, while the mass spectra obtained showed that BDMC contained CURC (relative abundance of 21%) and DMC (relative abundance of 17%). This curcuminoid was therefore found to be the least pure. Low-resolution ESI-MS show molecular ions of [M + 1] 369.2 amu for CURC, [M + 1] 339.2 amu for DMC, and [M + 1] 309.2 amu for BDMC. Experimental details and relevant spectra are included in the Supporting Information section.

Figure 1 shows that the co-incubation of primary hippocampal neurons with Pb (10 μM) significantly ($p < 0.001$) increases MDA levels; however co-incubation of the neurons with the curcuminoids reduces the lipid peroxidation in a concentration-dependent manner. The curcuminoids also improve the viability of the neurons (see **Figure 2**) that was significantly ($p < 0.001$) reduced by Pb (10 μM). However, BDMC fails to reduce lipid peroxidation at 50 μM while both DMC and BDMC fail to enhance neuron viability at the lower concentration (50 μM). CURC appears to be the most potent antioxidant of those tested.

The results (**Figure 3**) show a significant difference in latency to escape between the groups ($F_{4,45} = 21.3$, $p < 0.001$). Post hoc analyses (Scheffé tests) show that the latency to escape (over 14 days) of animals treated with Pb only was significantly longer compared to the control group ($p < 0.001$). Animals treated with CURC and DMC have significantly shorter escape times compared to the Pb-treated animals ($p < 0.001$). The latency

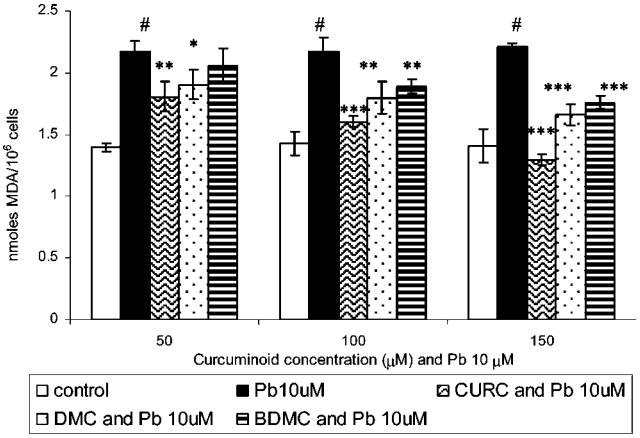

Figure 1. Effect of the curcuminoids on Pb (10 μM)-induced lipid peroxidation in primary hippocampal neurons. Each bar represents the mean ± SD ($n = 4$). $^{\#}p < 0.001$ as compared to controls. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ as compared to Pb (10 μM) groups. Results were analyzed using a one-way ANOVA followed by the Student–Newman–Keuls multiple range test.

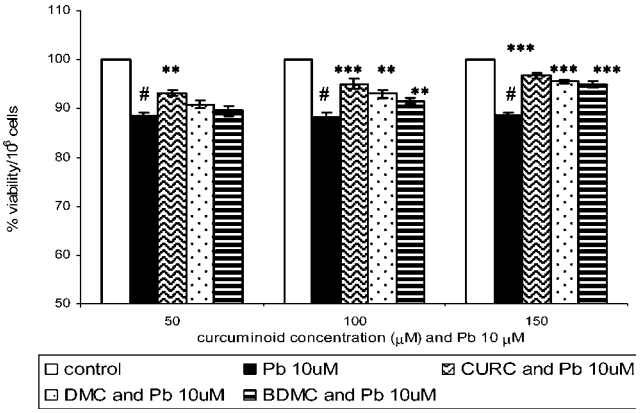

Figure 2. Effect of the curcuminoid and Pb (10 μM) on the viability of primary hippocampal neurons. Each bar represents the mean ± SD ($n = 4$). $^{\#}p < 0.001$ as compared to controls. $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared to Pb (10 μM) groups. Results were analyzed using a one-way ANOVA followed by the Student–Newman–Keuls multiple range test.

to escape of animals treated with BDMC show no significant difference compared animals treated with Pb only. There was no significant difference in the latency to escape between the animals treated with CURC and DMC.

Animals that were sacrificed immediately after the treatment regime (day = 1) and those that were dosed with Pb show a significant decrease in the amount of GSH in the rat hippocampus (**Figure 4**). However CURC and DMC, but not BDMC, prevent this decrease in the GSH content. Animals sacrificed at the end of the study (day = 14), show insignificant changes in the GSH content in rat hippocampus when compared to controls. The GSH content in Pb-only-dosed animals had improved ($p < 0.05$) when compared to controls.

As shown in **Figure 5**, animals treated with Pb and sacrificed immediately after the treatment regime had an increased amount of oxidized proteins compared to the control group. Treatment with CURC and DMC, but not BDMC, significantly reduces protein oxidation. All curcuminoid-treated animals sacrificed at the end of the behavioral studies show lower amounts of oxidized proteins compared to animals treated with Pb only.

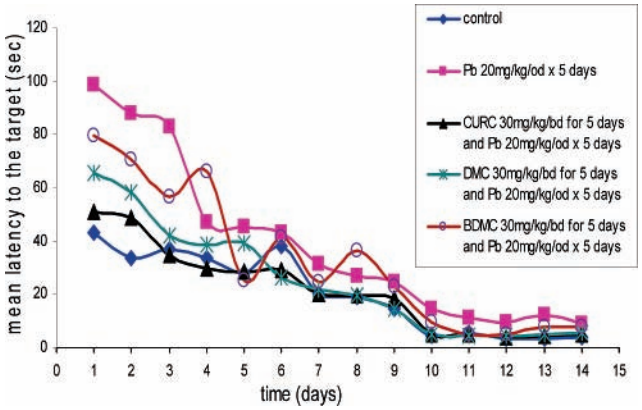

Figure 3. Performance in the water maze task of rats treated with Pb or with Pb and the curcuminoids. Trials were conducted after animals were injected for 5 days with Pb (20 mg/kg/od) or Pb (20 mg/kg/od) and curcuminoids (30 mg/kg/bd). Each point shows the average time taken for 10 rats. Trials were conducted once daily, every day for 14 days. Results were analyzed using repeated measures ANOVA with group as a factor and latency measured over 14 days. Scheffes' test for multiple group comparison was used for post hoc analysis.

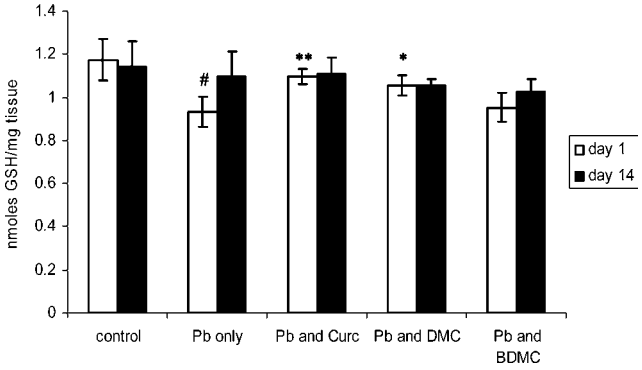

Figure 4. Effect of Pb alone or Pb and curcuminoid treatment on rat hippocampal glutathione content. Hippocampi from animals were analyzed after 5 days of treatment (day 1) or at the end of the behavioral studies (day 14). Each bar represents the mean ± SD ($n = 5$). $^{\#}p < 0.001$ in comparison to controls. $^{*}p < 0.05$ and $^{**}p < 0.01$ in comparison to Pb-only-dosed animals. Results were analyzed using a one-way ANOVA followed by the Student–Newman–Keuls multiple range test.

DISCUSSION

It has long been known that the hippocampus plays a key role in the formation of new memories and it has been extensively studied for both its role in learning and memory (*23*) as well as its susceptibility to excitotoxicity (*24*). Since hippocampal long-term potentiation is widely accepted as a form of neuronal plasticity underlying learning and memory, much attention was focused on the hippocampus in this study.

Lead has been shown to potentiate oxygen toxicity in rats. It accelerates the conversion of oxyhemeoglobin to methahemeoglobin which results in the production of superoxide and hydrogen peroxide (*25*). This heavy metal has also been shown to enhance the peroxidation of liposomes in the presence of hemeoglobin (*25*) and has also been shown to induce lipid peroxidation in rat brain homogenate (*16*). The present study shows that Pb induces lipid peroxidation in primary hippocampal neurons. However, the co-incubation of the neurons with the curcuminoids reduce the Pb-induced lipid peroxidation. It is thought that the methoxy group on the phenyl ring is one of the functional groups important for this compound's antioxidant property (*26*). CURC has two methoxy groups, DMC has one

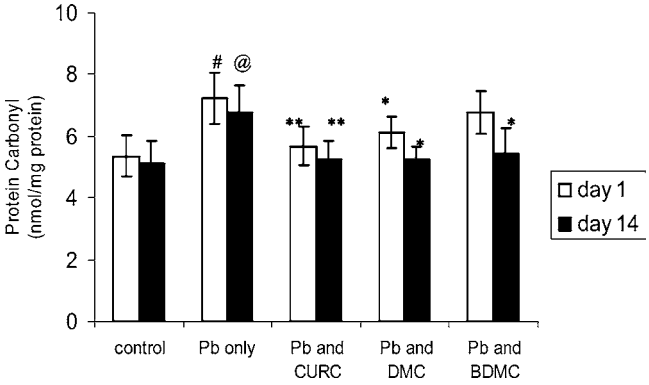

Figure 5. Effect of Pb alone or Pb and curcuminoid treatment on rat hippocampal protein carbonyl levels. Hippocampi from animals were analyzed after 5 days of treatment (day 1) or at the end of the behavioral studies (day 14). Each bar represents the mean ± SD ($n = 5$). $^{\#}p < 0.01$ and $^{@}p < 0.05$ in comparison to controls. $^{*}p < 0.05$ and $^{**}p < 0.01$ in comparison to Pb-only-dosed animals. Results were analyzed using a one-way ANOVA followed by the Student–Newman–Keuls multiple range test.

methoxy group, while BDMC has no methoxy groups. This could explain why CURC is more potent in reducing lipid peroxidation than BDMC.

Pb is thought to exert its toxicity by disrupting calcium-dependent mechanisms. Pb and calcium compete for the same binding sites on proteins belonging to a large family of ion binding proteins, and Pb often substitutes for calcium in such proteins (*27*). Evidence exists to support the hypothesis that calcium signaling is altered in Pb exposed rats (*28*). It has been shown that calcium (Ca^{2+}) influx during glutamate insults results in excitotoxicity (*29*) and subsequently cell death (*28*). Increased intracellular Ca^{2+} concentrations promote cell damage by both activating destructive enzymes and increasing the formation of reactive oxygen species (*30*). Pb could displace calcium at its binding sites and induce ROS production, which would explain the increase in lipid peroxidation and decreased cell viability. The curcuminoids scavenge free radicals and enhance cell viability. However, DMC and BDMC both fail at low concentrations to enhance cell viability. This suggests that these agents are not as potent free radical scavengers when compared to CURC at the same concentrations. It is also possible that the curcuminoids bind to Pb and prevent this heavy metal from inducing free radical generation and enhanced cell viability.

Pb crosses the blood−brain barrier and enters the brain with rapid kinetics (*31*). Studies have shown that Pb impairs neurobehavioral systems by reducing learning/memory capacities (*32*). The *N*-methyl-D-aspartate (NMDA) receptor is known to be directly involved in the plasticity phenomenon underlying learning and memory processes. It is thought that Pb-induced neurotoxicity such as memory deficits and intelligence deficiency results from Pb interfering with the NMDA receptors in the hippocampal formation and cerebral cortex (*33*). This study demonstrates that Pb significantly induced memory deficits in rats, and this was prevented or reduced by CURC and DMC treatment. The curcuminoids reduce the Pb-induced memory deficit by scavenging the free radicals generated by Pb and prevent or reduced the damage to neurons. It is also possible that the curcuminoids may bind Pb and prevent this heavy metal from interacting with the NMDA receptor since it has been shown (*16*) that CURC binds Pb and reduces Pb-induced neurotoxicity. BDMC, apart from being the weaker antioxidant, may have been bound weakly or not at all; this may also explain why it did not reduce the Pb-induced memory deficit.

The reduced form of the tripeptide GSH is the most abundant sulfur-containing antioxidant molecule in brain tissue. Lipid peroxides and hydrogen produced during the dismutation of the superoxide free radical are reduced by GSH peroxidase, converting GSH to its oxidized form. Imbalances in the expression of GSH and its related enzymes have been implicated in various pathological conditions which include cancer and neurodegenerative diseases (*34*). Exogenous agents, including those that enhance free radical generation, can also directly interact with intracellular reduced GSH and result in its depletion (*35*). It is also known that GSH binds to metals such as mercury (*36*) to prevent metal-induced neurotoxicity (*35*). This study shows that Pb decreases hippocampal GSH levels and that this decrease is curtailed by CURC and DMC treatment. The GSH levels returned to normal 14 days later after withdrawing Pb treatment. It is possible that the prooxidant/oxidant status of the cells have normalized. Apart from scavenging free radicals, the curcuminoids may also bind Pb and prevent GSH depletion. These data are in corroboration with previous research which also demonstrated that curcumin reduces GSH depletion and decreases Pb-induced lipid peroxidation in the rat brain (*37*).

It has been proposed (*38*) that protein oxidation is important in the development of various diseases and the aging process. Oxidative stress is known to enhance protein turnover accompanied by the removal of oxidized proteins (*39*). The removal of oxidized proteins has been considered to be impaired during the aging process (*40*). These results show that Pb increases protein oxidation in rat hippocampus. This was evident in the animals that were sacrificed immediately after the treatment protocol as well as after the behavioral studies (14 days later). It is also possible that Pb prevents or reduces the removal of oxidized proteins. These results also show that the curcuminoids CURC and DMC, but not BDMC, reduce the amount of oxidized proteins measured immediately after treatment in rat hippocampus. Protein carbonyl levels measured at the end of the study (14 days later) show that all curcuminoid-treated animals had less oxidized proteins. The antioxidant properties of the curcuminoids reduce the protein oxidation in a similar manner as these agents reduce lipid peroxidation.

It has been reported that the ideal treatment for Pb intoxication should encompass both chelating and antioxidant actions (*41*). The curcuminoids reduce Pb-induced lipid peroxidation in primary hippocampal neurons and enhance cell viability reduced by Pb. The curcuminoids reduce protein oxidation, lipid peroxidation, enhance cell viability, and reduce GSH depletion through their anti-oxidant activity. This prevents or reduces the loss of neurons in rat hippocampus and prevents the memory and learning deficit observed in animals that received Pb only. This study shows that CURC is the most potent antioxidant of the curcuminoids while BDMC is the weakest, implying that the methoxy group is involved in the curcuminoids' antioxidant activity. The neurotoxicity induced by Pb as well as the curcuminoids ability to reduce the neurotoxicity is demonstrated and suggests that turmeric from the *C. longa* plant has medicinal properties that may prevent or reduce neurodegenerative disorders and may be beneficial when used for heavy metal poisoning.

ABBREVIATIONS USED

Pb, lead; CURC, curcumin; DMC, demethoxycurcumin; BDMC, bisdemethoxycurcumin; GSH, glutathione; TBA, 2-thiobarbituric acid; BHT, butylated hydroxytoluene; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DTNB, 5,5′-dithiobisnitrobenzoic acid; TCA, trichloroacetic acid; MEM, Eagle's Minimum Essential Medium; FCS, fetal calf serum; TLC, thin-layer chromatography; MDA, malondialdehyde; PBS, phosphate buffered saline; ANOVA, analysis of variance.

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Supporting Information Available: TLC, NMR, and MS data used to determine the purity of the curcuminoids. This material is available free of charge via the Internet at http://pubs.acs.org.

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