

Protective Effect of Curcumin on Anxiety, Learning Behavior, Neuromuscular Activities, Brain Neurotransmitters and Oxidative Stress Enzymes in Cadmium Intoxicated Mice

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ABSTRACT

Cadmium (Cd) exposure can induce acute lethal health-related threats to humans since it has an exceptional ability to accumulate in living organisms and cause toxicological effects. Curcumin (Cur) on the other hand has a wide variety of biological activities and several animal studies have suggested for a potential therapeutic or preventive effects against several ailments and infections. To study the effect of Cur on the toxicity of Cd, sixty Swiss-Webster strain male mice were divided into 6 groups of ten each at random. Group-1 served as the naïve control and received no treatment. Group-2, 3 and 4 were the experimental controls and were administered once a day with a single oral dose of 50% dimethyl sulphoxide (DMSO), Cur (300 mg/kg) or Cd (100 mg/kg) respectively, for 2 weeks. Group-5 and 6 received Cur and Cd in combination once a day orally for 2 weeks except that Cur in a dose of 150 and 300 mg/kg to group 5 and 6 respectively, was administered one hour before Cd (100 mg/kg) administration to both groups. After treatment period, the animals were subjected to behavioral tests and thereafter, the animals were sacrificed for the estimation of neurotransmitters like serotonin (5-HT), dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) as well as oxidative stress enzymes like lipid peroxides in the form of thiobarbituric acid-reactive substances (TBARS) and total glutathione (GSH) in the forebrain tissue. Cd reduced significantly the body weight gain, the locomotor activity, anxiety behavior in the plus maze and the learning capability (cognitive effect) in the shuttle-box test. Biochemical analysis further revealed that Cd exposure significantly altered the brain neurotransmitters and the oxidative stress enzymes. However, administration of Cur along with Cd had an ameliorating effect on all the behavioral and biochemical parameters studied herein and reduced the toxicity of Cd significantly and dose-dependently. Thus, Cur may be beneficial for anxiety, neuromuscular, and cognitive problems and protect from Cd intoxication.

Keywords: Curcumin; Cadmium; Male Mice; Anxiety; Cognitive Behaviors; Neurotransmitters; Oxidative Stress

1. Introduction

Cadmium (Cd) represents one of the most toxic and carcinogenic heavy metal [1]. It is considered as a serious environmental and industrial pollutant and may represent as a serious health hazard to humans and other animals [2-4]. Some important sources of Cd exposure for humans can be emissions from industries of batteries, metal plating, pigments, plastics, toys and alloy, cigarette smoking and through dietary consumption [5-7]. Exposure to Cd may cause lesions in many organs such as the liver, kidney and testis [8-11], leading to various possible pathological conditions such as hepatic, renal and testicular

dysfunction, respiratory and nervous system disorders [12,13]. Cd is reported to induce the generation of reactive oxygen species (ROS), and this oxidative stress was found to result in mitochondrial dysfunction and apoptosis, both *in vivo* and *in vitro* [14,15]. The oxidative damage within the tissues and DNA damages is considered to be an early manifestation of Cd toxicity and carcinogenicity [16,17].

Curcumin (Cur) is a well known biologically active natural phytochemical phenolic compound (diferuloyl methane) found as a major component in turmeric, a yellow curry spice, extracted from the rhizome of *Curcuma longa* L. (family Zingiberaceae). Cur is well absorbed in the body system and has exceedingly low toxicity [18]. It

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possesses many beneficial activities in the body and is effective in several disorders including anorexia, coryza, cough, hepatic diseases, and sinusitis [19,20]. Recent studies provide scientific evidence regarding the potential pharmacological, prophylactic or therapeutic use of Cur, as anti-inflammatory, anticarcinogenic, antiviral, antifungal, antiparasitic, antimutagen, antiinfectious and antioxidant compound [21-26]. The multiple beneficial effects of Cur have also been elaborated in neurogenesis process which in turn has been reported for its neuroprotective effects in age-related neurodegenerative diseases [27]. Several studies have shown that Cur exhibits protective effects against oxidative damage and has antioxidant property exerting powerful oxygen free radical scavenging effects and increased intracellular glutathione concentration, thereby protecting lipid peroxidation [28-32]. Commercial Cur contains 77% curcumin, 17% demethoxycurcumin and 3% bisdemethoxycurcumin [33] and virtually all these three components in Cur are biologically active and possess protective properties [34].

In the light of the above information it appears that Cur may prove beneficial in several ways for Cd toxicities and this aspect needs more and more research work. Thus, the present study was undertaken to explore the effects of Cur against the Cd induced behavioral deficits and biochemical toxicity in the brain of male mice.

2. Materials and Methods

2.1. Experimental Animals

Sixty male Swiss-Webster strain mice (8 - 10 weeks old) were housed in opaque plastic cages under hygienic conditions in the animal facility of the Zoology Department, King Saud University, Riyadh, Saudi Arabia. All animals were maintained under reversed lighting conditions with white lights on from 22.00 to 10.00 hours local time. The ambient temperature was regulated between 20°C and 22°C. Food (Pilsbury's Diet) and water were available *ad libitum*, unless otherwise indicated. All procedures were carried out in accordance with the ethical guidelines for care and use of laboratory animals, and all protocols were approved by the local Ethics and Care of Experimental Animals Committee.

All animals were divided into six different groups with ten animals in each. Group I consisted of untreated mice and served as naïve controls. Group II was treated with 50% DMSO (solvent of Cur). Group III was treated with 300 mg/kg Cur dissolved in 50% DMSO. Group IV was treated with Cd (100 mg/kg). Groups V and VI consisted of mice administered with Cur as well as Cd in combination in the doses of 150 + 100 and 300 + 100 mg/kg respectively. All exposures were through oral administration, once a day, for two weeks, except that in groups V

and VI, Cur was administered one hour before Cd exposure.

2.2. Cur and Cd Administration

Cur of analytical grade, Sigma Chemical Company, USA, was dissolved in 50% DMSO to give a dose of 150 and 300 mg/kg body weight and diluted further with drinking water in 1.0 ml volume and was administered orally once a day. Cd was also administered orally once a day in the form of cadmium chloride (analytical grade, Riedel de Haen, Germany) dissolved in drinking tap water at a dose of 100 mg/kg body weight in 1.0 ml volume. In the fifth and sixth groups of animals where Cur and Cd were administered together orally once a day, Cur was administered one hour before Cd administration. The naïve control group received 1.0 ml plain tap water only. The doses of Cur and Cd used in this study are at par with the effective doses reported in the literature for such studies. The factor for the possibility of presence of Cd traces in food and tap water was not taken into account for calculating the daily Cd intake. However, this factor was minimized by giving the same source of food and tap water to all experimental groups including the controls.

2.3. Body Weight

The body weight of all animals from each experimental group was recorded on day 1 of the treatment and on days 5, 10 and 15 of the total treatment period. Thereafter, the animals were subjected to behavioral tests and subsequently were sacrificed for the isolation of fore-brain tissue for the biochemical estimations.

2.4. Behavioral Studies

Anxiety, learning capability and locomotor behavior in all animals were measured in the same order in plus maze, shuttle-box and in automated activity meter respectively.

2.4.1. Anxiety Behavior in the Elevated Plus-Maze Test

The elevated plus-maze (with 2 opened and 2 enclosed arms) is frequently used as a measure for evaluating the risk assessment and anxiety behavior of an ethologically derived animal model [35]. The plus-maze was elevated to a height of 80 cm above the floor. The mice were individually placed onto the central platform facing one of the open arms and were observed for 5 min while freely exploring the maze. The animal was considered to have entered an arm when all four limbs were inside the arm. Duration of time spent in open and enclosed arms and number of entries in open and enclosed arms were measured during the test period. On completion of the test, the maze was cleaned with a 10% ethanol solution to control

for any possible olfactory cues.

2.4.2. Learning Capability in the Shuttle-Box Test (Active Avoidance Responses)

The active avoidance responses were measured in the animals using an automatic reflex conditioner "shuttle box" (Ugo Basile, Comerio-Varese, Italy). The rectangular shaped shuttle-box was divided into two chambers of equal size by a stainless steel partition with a gate providing access to the adjacent compartment. Before starting the trial sessions, each animal was allowed to adapt and acquaint itself with the shuttle box for 2 min without any stimulus. A light bulb (21 W) for 6 s duration and a buzzer (670 Hz and 70 dB) was switched on consecutively and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s. The US was an electric scrambler shock (1 mA for 4 s) applied to the metallic grid floor. If the animal avoided the US by running into the other compartment within 5 s after the onset of the CS, the microprocessor recorder unit of the shuttle box recorded an avoidance response and this was considered as conditioned avoidance response to avoid the electric shock. Each animal was given 50 trials with a fixed inter trial interval of 15 s. During the 50 trials session of the individual animal, the total number of avoidance was measured. The total time taken until the animal entered the other compartment to avoid the shock treatment (latency of avoidance response or escape latency in seconds) was also measured for each animal. The recorder unit of the automated shuttle box continuously recorded these parameters during the whole experimental period (50 trials) of each animal.

2.4.3. Motor Activity Test in Automated Activity Meter

Motor activity was measured using automated electronic activity meter (Ugo Basile, Comerio-Varese, Italy). The horizontal and vertical motor activities were detected by arrays of infrared beams located above the floor of the testing arena. Each interruption of the beams on the x or y axis generated an electric impulse which was recorded on a digital counter. Each animal was tested separately and the motor activity was recorded for a period of 2 min in the activity meter.

2.5. Biochemical Studies

Immediately after completing the behavioral tests, the animals were sacrificed by decapitation, the brains were dissected on ice, the fore brain areas (containing the hippocampus and striatum areas) were removed and frozen in liquid nitrogen and stored at -70°C for determination of monoamines, lipid peroxides (TBARS) and glutathione content.

2.5.1. Determination of Monoamines

The monoamines were estimated using the modified method of Patrick *et al.* [36]. A 10% homogenate of fore brain tissue was prepared by homogenizing the tissues for 10 s in 0.1 M HClO_4 containing 0.05% EDTA, centrifuged at 17,000 rpm at 4°C for 5 min. The supernatants were filtered using $0.45\ \mu\text{m}$ pore filters and analyzed by high performance liquid chromatography (HPLC). The mobile phase consisted of 32 mM citric acid monohydrate, 12.5 mM disodium hydrogen orthophosphate, 7% methanol, 1 mM octane sulphonic acid and 0.05 mM EDTA. The mobile phase was filtered through $0.22\ \mu\text{m}$ filter and degassed under vacuum before use. $\mu\text{Bondpak C18}$ column was used at a flow rate of 1.2 ml/min and the injection volume of the sample was 20 μl . The levels of dopamine (DA), DOPAC and serotonin or 5-hydroxytryptamine (5-HT) were calculated using a calibration curve and results were expressed as ng/mg tissue weight.

2.5.2. Determination of Lipid Peroxides

Lipid peroxides (LP) in fore brain tissue were determined spectrophotometrically as thiobarbituric acid-reactive substances (TBARS) according to the method of Ohkawa *et al.* [37]. The tissue samples were homogenized in 1.15% cold KCl with an Ultraturax homogenizer. After centrifugation at $3000\times\ \text{g}$ for 5 min, an aliquot of supernatant was mixed with 2 ml of reaction mixture (containing 15% trichloroacetic acid and 0.375% thiobarbituric acid solution in 0.25 N HCl) and heated for 5 min in a boiling water bath. The tubes were cooled at room temperature and centrifuged at $1000\times\ \text{g}$ for 10 min. The absorbance of supernatant was read at 535 nm against a blank that contained all reagents except homogenate. Tissue lipid peroxide levels were quantified using extinction coefficient of $1.56 \times 10^5\ \text{m}^{-1}\cdot\text{cm}^{-1}$ and expressed as nanomoles of TBARS formed per g tissue weight.

2.5.3. Determination of Glutathione

Total glutathione (GSH) level in fore brain tissue was measured enzymatically in the brain tissues by a slightly modified method of Mangino *et al.* [38]. Briefly, about 50 mg of isolated brain tissues were homogenized with 1 ml 0.1 M perchloric acid plus 0.005% EDTA. The homogenates were centrifuged at 4000 rpm for 10 min and the supernatants were used for GSH assay. The reaction mixture consisted of the following three freshly prepared solutions: solution I, 0.3 mM NADPH; solution II, 6 mM 5,5'-dithio-bis(2-nitrobenzoic acid) and solution III, 50 U/ml glutathione (all chemicals from Sigma). All three solutions were prepared with a stock buffer consisting of 125 mM NaH_2PO_4 and 6.3 mM EDTA at pH 7.5. At the time of glutathione assay, 800 μl of solution I, 100 μl of solution II, and 10 μl of solution III were mixed in a quartz cuvette and placed in a dual beam UV-VIS spec-

trophotometer (Shimadzu UV160) at 30°C. The enzymatic reaction was started by the addition of 100 µl of the supernatant and the absorbance was monitored for 3 min at 412 nm. The slope of the change in absorbance was used for quantitative estimation of total GSH by comparing the slope of the samples with a standard curve prepared with pure glutathione (Sigma).

2.6. Statistical Analysis

The data were analyzed for variance (Bartlett's test for equal variance) and normality (Gaussian-shaped distribution) using the Kolmogorov-Smirnov goodness-of-fit test. As the data passed the normality test ($p > 0.10$), group means were compared with the ANOVA with post-hoc testing using Tukey-Kramer Multiple Comparisons Test or Student-Newman-Keuls Multiple Comparisons Tests. All results were expressed as means \pm SEM and the significance were defined as $p < 0.05$ for all tests.

3. Results

3.1. Body Weight

Exposure to Cd for two weeks showed a significant ($p < 0.001$) depletion in the body weight gain of the treated rats whereas Cur alone showed no significant changes in the body weight as compared to the control animals. However, significant and dose-dependent ameliorating effect of Cur was found in Cd-induced alterations in the body weight gain when the animals were treated with Cd and Cur in combination (Figure 1).

3.2. Behavioral Studies

3.2.1. Elevated Plus-Maze Test

The time spent in open arms was significantly ($p < 0.001$) lower, whereas time spent in enclosed arms was significantly ($p < 0.001$) higher in animals treated with Cd as compared to controls (Figure 2(a)). The number of entries in open arms was significantly ($p < 0.001$) lower and in enclosed arms was significantly ($p < 0.001$) higher in Cd treated groups exhibiting more anxiety related exploratory activity as compared to controls (Figure 2(b)). Cur alone had no effects in any of the parameters as compared to the controls (Figures 2(a) and (b)). However, in the Cur and Cd combination treated group, Cur pretreatment significantly ($p < 0.01$) and dose-dependently attenuated the Cd induced anxiety and behavioral abnormalities (Figures 2(a) and (b)).

3.2.2. Shuttle-Box Test

In the shuttle-box active avoidance test, the Cd-exposed animals, showed a statistically significant ($p < 0.001$) decrease in the number of avoidances during the trial period as compared to the control group (Figure 3(a)). The total

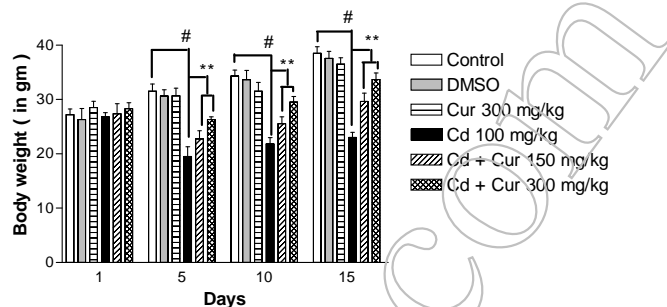


Figure 1. Ameliorating effect of curcumin on the declining body weight gain in the cadmium treated mice. * represents statistically significant ($p < 0.001$) from the control group whereas # and ## represent significantly different ($p < 0.05$ and $p < 0.01$ respectively) from the cadmium treated group by ANOVA and student's t-test.**

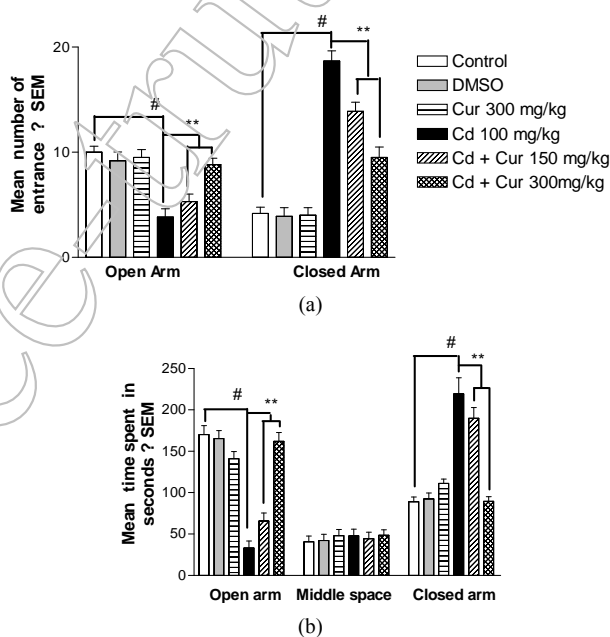


Figure 2. Protective effect of curcumin on the cadmium-induced anxiety in the mice measured in a plus maze activity meter by estimating the total time spent (a) and the number of entries (b) in the open and enclosed arms of the plus maze. * represents statistically significant ($p < 0.001$) from the control group whereas ## and ### represents significantly different ($p < 0.01$ and $p < 0.001$ respectively) from the cadmium treated group by ANOVA and student's t-test.**

time taken during the entire trials by the Cd treated animals to enter the other compartment to avoid the shock treatment (latency of avoidance or escape latency response in seconds) was significantly ($p < 0.001$) greater as compared to the controls (Figure 3(b)). Animals exposed to Cd were poor learners and took significant time in avoiding the shock treatment as compared to the controls (Figures 3(a) and (b)). Cur alone had no effect on the active avoidance performances, however, Cur in combination with Cd showed a significant ($p < 0.05$) and

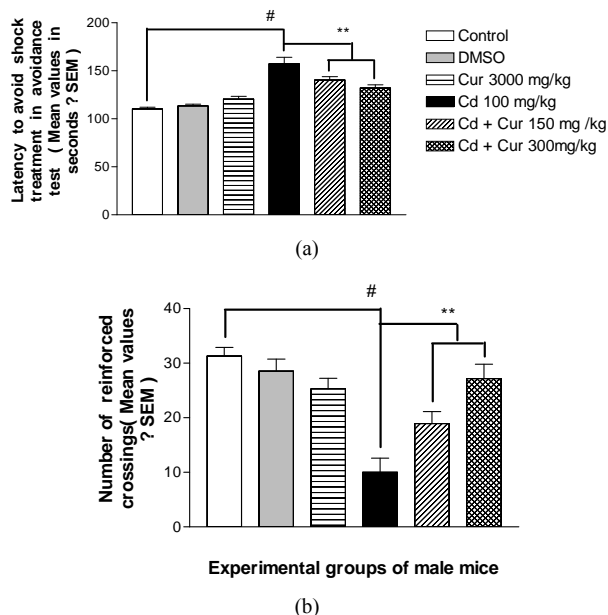


Figure 3. Protective effects of curcumin on the cadmium-induced cognitive (learning) performance in shuttle box test for the mice to take time (latency) in avoiding the shock treatment (a) and the number of reinforced crossing the chamber (b) for avoiding the shock treatment during stimulus of light and sound. * represents statistically significant ($p < 0.001$) from the control group whereas # and ## represent significantly different ($p < 0.05$ and $p < 0.01$ respectively) from the cadmium treated group by ANOVA and student's *t*-test.**

dose-dependent attenuating effect of Cur pretreatment on the Cd-induced poor learning capabilities (Figures 3(a) and (b)).

3.2.3. Motor Activity

Treatment with Cd significantly affected the vertical as well as the horizontal motor activity ($p < 0.001$) as compared to the control (Figure 4). Cur alone had no significant effect on these activities but concomitant treatment with Cur and Cd significantly ($p < 0.01$) and dose-dependently attenuated the Cd-induced motor impairment (Figure 4).

3.3. Biochemical Studies

3.3.1. Levels of Monoamines in Forebrain Tissue

There was a significant ($p < 0.001$) depletion of DA and DOPAC in the forebrain areas of mice treated with Cd as compared to the control group (Figures 5(a) and (b) respectively). Similarly, there was a significant ($p < 0.001$) depletion of 5-HT in the forebrain tissue of the Cd treated group as compared to the control (Figure 5(c)). Exposure to Cur alone had no alteration in the levels of these neurotransmitters as compared to the controls. However, in the group that was administered Cur and Cd in combina-

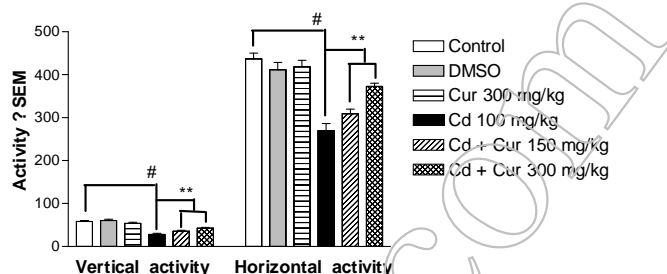


Figure 4. Attenuating effect of curcumin on the declining locomotor (horizontal and vertical) activity of mice treated with cadmium measured in electronic activity meter. * represents statistically significant ($p < 0.001$) from the control group whereas # and ## represent significantly different ($p < 0.05$ and $p < 0.01$ respectively) from the cadmium treated group by ANOVA and student's *t*-test.**

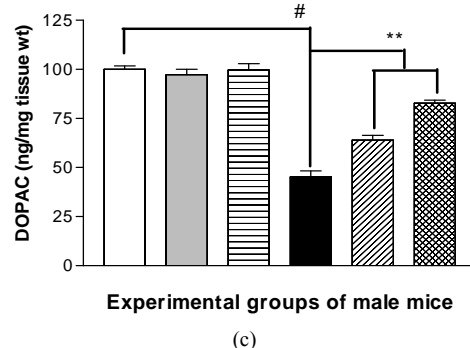
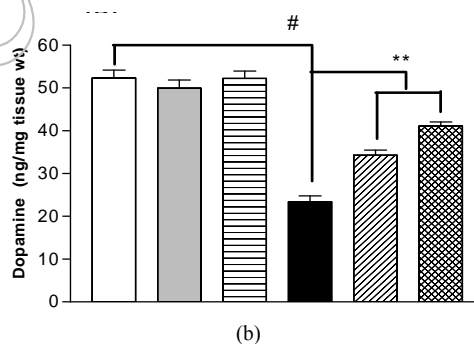
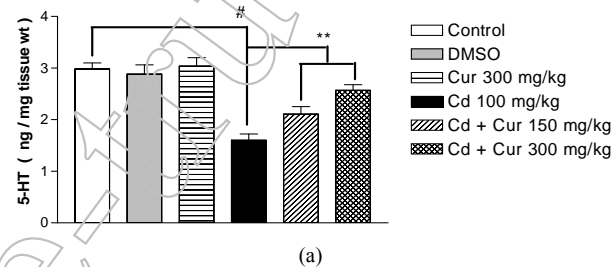


Figure 5. Ameliorating effect of curcumin on the depleting levels of the neurotransmitters like (a) serotonin (5-HT), (b) dopamine (DA) and (c) the byproduct of DA (DOPAC), due to cadmium treatment in the fore brain area of the male mice. * represents statistically significant ($p < 0.001$) from the control group whereas # represents significantly different ($p < 0.05$) from the cadmium treated group by ANOVA and student's *t*-test.**

tion, pretreatment of animals with Cur, significantly ($p < 0.001$) and dose-dependently attenuated Cd-induced depletion of DA and DOPAC (Figures 5(a) and (b) respectively) and 5-HT (Figure 5(c)) in the forebrain tissue as compared to the Cd treated groups.

3.3.2. Lipid Peroxidation (TBARS) Levels in the Forebrain Tissue

The lipid peroxidation (TBARS) level in the forebrain tissues (Figure 6(a)) were markedly ($p < 0.001$) increased in the Cd treated group as compared to the control. Cur alone had no effect on the level of TBARS, however, in the Cur and Cd combination group, Cur pre-treatment significantly ($p < 0.05$) and dose-dependently attenuated Cd-induced increase in TBARS level (Figure 6(a)) as compared to the Cd group.

3.3.3. Glutathione (GSH) Levels in the Forebrain Tissue

A highly significant ($p < 0.001$) depletion in the GSH level was observed in the forebrain tissue of Cd treated group (Figure 6(b)). However, Cur alone had no alteration on the normal level of GSH. In the combination group (Cur + Cd), Cur pre-treatment significantly ($p < 0.05$) and dose-dependently attenuated the Cd-induced depletion of GSH in the forebrain tissue (Figure 6(b)) as compared to Cd group.

4. Discussion

The present results suggest that exposure of male mice to Cd is toxic and influences various behavioral activities as well as the levels of enzyme activities in the brain tissues of the treated animals. The rodents exposed to Cd in earlier studies also are reported to display lowered body weight [39,40], impaired behavioral activity and worsened conditioned reflex response [41,42], and impaired neurobehavioral [43] and neurotoxicological [42,44] developments. It is therefore likely that the above factors may singly or together ultimately produce behavioral effects of Cd [45] in the present study also.

The major target organs that are reported for the acute oral toxicity of Cd are liver [46] and central nervous tissue [47]. Cd has been recognized as one of the most toxic environmental and industrial pollutants that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues. A significantly increased accumulation of Cd in liver, kidneys and other organs have been reported with the severity of their intoxication dependent on the route, dose, and duration of the exposure to the metal [48-50]. Previous investigations show that oral intake of Cd induces its accumulation in the red blood cells [51], heart [52] and skeletal muscle of rats [53], which was accompanied by considerable alterations of enzymatic and non-enzymatic component of antioxi-

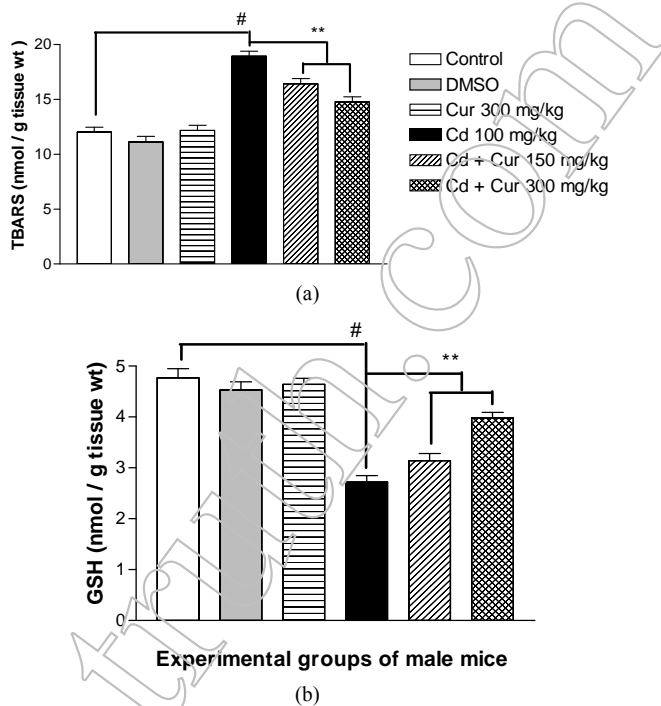


Figure 6. Protective effect of curcumin on the cadmium-induced oxidative stress depicted by increased level of TBARS (a) and decreased level of GSH (b) in the forebrain of the mice. * represents statistically significant ($p < 0.001$) from the control group whereas # represents significantly different ($p < 0.05$) from the cadmium treated group by ANOVA and student's t-test.**

dant defense system (AOS). At cellular level also it has been reported that, Cd mainly accumulates in the cytosol (70%), followed by the nucleus (15%) and lowest in mitochondria and the endoplasmic reticulum [54]. Such accumulation of Cd mainly in cytosol might have lead to variations in the phosphate pool of the animals which ultimately lead to disturbed energy source with consequent disturbance in their metabolism [55], and this is probably reflected in the form of disturbed behavioral activities.

The results of the present study showed that the levels of neurotransmitters DA, DOPAC and 5-HT were depleted significantly by Cd treatment in the forebrain (cerebral part containing hippocampus and striatum) tissue of the Cd-exposed mice. There is evidence of an inhibitory role of DA mediated receptor (D_2 type) in depressing the hyperexcitability of hippocampal and striatal neurons [56, 57]. A number of 5-HT receptor subtypes have been reported for having different roles in the functions of serotonergic neurotransmission, including the functions connected with learning and memory processes [58]. The mice exposed to Cd performed badly in plus maze parameters and also resulted in decreased number of avoidances (escapes) in the automatic reflex conditioner as compared to the control animals. This suggests for a tendency towards decreasing of the exploratory and memory

effect of Cd under conditions of reduced functional capacity of serotonergic neurotransmission as also reported earlier for aluminum toxicity [59]. Recently, a growing body of research has focused on the participation of serotonin (5-HT) in the neurochemical mechanisms of cognition and especially of learning and memory. Potential toxic mechanisms of action for Cd may include disruption in serotonergic neurotransmission through disturbed levels of neurotransmitters in the brain hippocampus [60].

Other studies have also shown that Cd inhibits the activity of majority of enzymes involved in AOS [61-63] inducing an increased production of free radicals, lipid peroxidation, and destruction of cell membranes [51,54,64]. Cd is also reported to inhibit the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis [65,66].

Cur in the present study had a significantly ameliorating effect on the Cd-induced deficits in the body weight, anxiety behavior, learning capability (cognitive effect) and muscular activity. Biochemical analysis in forebrain tissue also revealed that Cur significantly attenuated Cd-induced neurotransmitters (reportedly associated with locomotor and cognitive activities) and the Cd-induced oxidative stress related enzymes (associated with behavioral and cognitive deficits). Furthermore, the ineffectiveness of Cur alone to cause any behavioral and biochemical deficits, clearly suggest that Cur alone is non-toxic and further supports for the ameliorating effect of Cur on the behavioral and biochemical toxicity induced by Cd. The biochemical damage may be due to the fact that Cd induces an oxidative stress that results in oxidative deterioration of biological macromolecules [40,67]. Cur reportedly has potent antioxidant activities [68,69], anti-inflammatory [70] and chemoprotective properties [71]. It has been shown to have a neuroprotective effect in models of cerebral ischemia [72,73], ethanol induced brain damage [74] and reduced amyloid pathology in transgenic mice of Alzheimer's disease [75].

Lipid peroxidation (LP) is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics [76,77]. Our results confirm that intoxication with Cd causes a significant increase of lipid peroxide concentration in forebrain tissue of mice. Since it causes LP in numerous tissues both *in vivo* and *in vitro* [6,51,62,65], it has been suggested that Cd may induce oxidative stress by producing hydroxyl radicals [78], superoxide anions, nitric oxide and hydrogen peroxide [79,80]. Moreover, it has been shown that various antioxidants and AOS protect cells from Cd-induced toxicity [81-83].

Co-treatment with Cur in the present study was effective in the prevention of oxidative damage induced by Cd, which resulted in significantly lower lipid peroxides con-

centration in the form of TBARS in the forebrain tissue. This can be explained by the important role of Cur in preventing LP and in protection of integrity and functioning of tissues and cells. The prevention of LP is essential for all aerobic organisms and so the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals [84]. The role of antioxidants in reversing this oxidative stress has been a matter of deep interest to basic scientists and clinicians [85].

The decreased activity of GSH due to Cd exposure in the present study suggests for the disturbed oxidant and antioxidant system in the brain tissue. Furthermore, presence of Cur along with Cd ameliorates significantly the GSH level and tries to increase the level of GSH indicating for the role of Cur as an antioxidant. It has been well established that the antioxidants such as Vit E, Vit C and GSH protect the membrane from oxidative damage [64,84,85]. In the present study, Cur reduced the cellular toxicity caused by Cd-induced ROS and protected the brain antioxidant system. Thus, Cd accumulation in brain tissue is most likely due to chronic dietary intake of Cd, and it is associated with marked alteration of neurotransmitters and enzyme GSH of AOS. These results also suggest that LP is associated with Cd toxicity in brain tissue. Our results showed that the antioxidant Cur ameliorated oxidative stress and loss of cellular antioxidants and suggested that Cur efficiently protect forebrain from Cd-induced oxidative damage. However, for asserting this statement, further studies are needed to measure other enzymatic components like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione-S transferase (GST) and nonenzymatic components such as vitamin C (Vit C) and vitamin E (Vit E) of AOS.

5. Conclusion

It is concluded from the present study that although Cur has many possible reported benefits, the full effects however, are not yet fully understood and more and more research work is needed. The results indicate that Cur possesses several multifold beneficial effects that may include better cognitive performance, better muscular activity and protection from externally induced oxidative stress and neurotransmitters dysfunction in the brain. Thus, inclusion of Cur in our normal diet and its use as a nutritional supplement may have a tremendous potential for health improvement and protection from Cd intoxication.

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REFERENCES

- [1] IARC, "International Agency for Research on Cancer Monographs. Cadmium," IARC Press, Lyon, 1993, pp. 119-238.
- [2] M. M. Brzoska and J. M. Jakoniuk, "Interactions between Cadmium and Zinc in the Organism," *Food and Chemical Toxicology*, Vol. 39, No. 10, 2001, pp. 967-980. [doi:10.1016/S0278-6915\(01\)00048-5](https://doi.org/10.1016/S0278-6915(01)00048-5)
- [3] M. M. Brzoska, K. Majewska and E. Kupraszewicz, "Effects of Low, Moderate and Relatively High Chronic Exposure to Cadmium on Long Bones Susceptibility to Fractures in Male Rats," *Environmental Toxicology and Pharmacology*, Vol. 29, No. 3, 2010, pp. 235-245. [doi:10.1016/j.etap.2010.01.005](https://doi.org/10.1016/j.etap.2010.01.005)
- [4] J. L. Li, R. Gao, S. Li, J. T. Wang, Z. X. Tang and S. W. Xu, "Testicular Toxicity Induced by Dietary Cadmium in Cocks and Ameliorative Effect by Selenium," *Biomaterials*, Vol. 23, No. 4, 2010, pp. 695-705. [doi:10.1007/s10534-010-9334-0](https://doi.org/10.1007/s10534-010-9334-0)
- [5] P. B. Hammond and E. C. Foulkes, "Metal Ion Toxicity in Man and Animals," In: H. Sigel, Ed., *Metal Ions in Biological Systems*, Marcel Dekker, New York, 1986, pp. 157-200.
- [6] F. M. El-Demerdash, M. I. Yousef, F. S. Kedwany and H. H. Baghdadi, "Cadmium-Induced Changes in Lipid Peroxidation, Blood Hematology, Biochemical Parameters and Semen Quality of Male Rats: Protective Role of Vitamin E and Beta-Carotene," *Food and Chemical Toxicology*, Vol. 42, No. 10, 2004, pp. 1563-1571. [doi:10.1016/j.fct.2004.05.001](https://doi.org/10.1016/j.fct.2004.05.001)
- [7] P. F. de Souza, M. A. Diamante and H. Dolder, "Testis Response to Low Doses of Cadmium in Wistar Rats," *International Journal of Experimental Pathology*, Vol. 91, No. 2, 2010, pp. 125-131. [doi:10.1111/j.1365-2613.2009.00692.x](https://doi.org/10.1111/j.1365-2613.2009.00692.x)
- [8] M. Satoh, H. Koyama, T. Kaji, H. Kito and C. Tohyama, "Perspectives on Cadmium Toxicity Research," *Tohoku Journal of Experimental Medicine*, Vol. 196, No. 1, 2002, pp. 23-32. [doi:10.1620/tjem.196.23](https://doi.org/10.1620/tjem.196.23)
- [9] R. A. Goyer, J. Liu and M. P. Waalkes, "Cadmium and Cancer of Prostate and Testis," *Biomaterials*, Vol. 17, No. 5, 2004, pp. 555-558. [doi:10.1023/B:BIOM.0000045738.59708.20](https://doi.org/10.1023/B:BIOM.0000045738.59708.20)
- [10] L. C. Xu, H. Sun, S. Y. Wang, L. Song, H. C. Chang and X. R. Wang, "The Roles of Metallothionein on Cadmium-Induced Testes Damages in Sprague Dawley Rats," *Environmental Toxicology and Pharmacology*, Vol. 20, No. 1, 2005, pp. 83-87. [doi:10.1016/j.etap.2004.10.008](https://doi.org/10.1016/j.etap.2004.10.008)
- [11] S. Amara, H. Abdelmelek, C. Garrel, P. Guiraud, T. Douki, J. L. Ravanat, A. Favier, M. Sakly and K. Ben Rhouma, "Preventive Effect of Zinc against Cadmium-Induced Stress in the Rat Testis," *Journal of Reproductive Development*, Vol. 54, No. 2, 2008, pp. 129-134. [doi:10.1262/jrd.18110](https://doi.org/10.1262/jrd.18110)
- [12] J. Thompson and J. Bannigan, "Cadmium: Toxic Effects on the Reproductive System and the Embryo," *Reproductive Toxicology*, Vol. 25, No. 3, 2008, pp. 304-315. [doi:10.1016/j.reprotox.2008.02.001](https://doi.org/10.1016/j.reprotox.2008.02.001)
- [13] B. I. Ognjanovic, S. D. Markovic, N. Z. Ethordevic, I. S. Trbojevic, A. S. Stajn and Z. S. Saicic, "Cadmium-Induced Lipid Peroxidation and Changes in Antioxidant Defense System in the Rat Testes: Protective Role of Coenzyme Q(10) and Vitamin E," *Reproductive Toxicology*, Vol. 29, No. 2, 2010, pp. 191-197. [doi:10.1016/j.reprotox.2009.11.009](https://doi.org/10.1016/j.reprotox.2009.11.009)
- [14] S. Jimi, M. Uchiyama, A. Takaki, J. Suzumiya and S. Hara, "Mechanisms of Cell Death Induced by Cadmium and Arsenic," *Annals of New York Academy of Sciences*, Vol. 1011, No. 1, 2004, pp. 325-333. [doi:10.1196/annals.1293.032](https://doi.org/10.1196/annals.1293.032)
- [15] R. Sen Gupta, J. Kim, C. Gomes, S. Oh, J. Park, W. B. Im, J. Y. Seong, R. S. Ahn, H. B. Kwon and J. Soh, "Effect of Ascorbic Acid Supplementation on Testicular Steroidogenesis and Germ Cell Death in Cadmium-Treated Male Rats," *Molecular and Cellular Endocrinology*, Vol. 221, No. 1-2, 2004, pp. 57-66. [doi:10.1016/j.mce.2004.03.012](https://doi.org/10.1016/j.mce.2004.03.012)
- [16] M. P. Waalkes, T. P. Coogan and R. A. Barter, "Toxicological Principles of Metal Carcinogenesis with Special Emphasis on Cadmium," *Critical Review of Toxicology*, Vol. 22, No. 3-4, 1992, pp. 175-201. [doi:10.3109/10408449209145323](https://doi.org/10.3109/10408449209145323)
- [17] M. P. Waalkes, "Cadmium Carcinogenesis in Review," *Journal of Inorganic Biochemistry*, Vol. 79, No. 1, 2000, pp. 241-244. [doi:10.1016/S0162-0134\(00\)00009-X](https://doi.org/10.1016/S0162-0134(00)00009-X)
- [18] National Toxicology Program, "NTP Toxicology and Carcinogenesis in Studies of Turmeric Oleoresin (CAS No. 8024-37-1) (Major Component 79% - 85% Curcumin, CAS No. 458-37-7) in F344/N Rats and B6C3F1 Mice (Feed Studies)," *National Toxicology Program Technical Reproductive Services*, Vol. 427, 1993, pp. 1-275.
- [19] I. Rahman, S. K. Biswas and P. A. Kirkham, "Regulation of Inflammation and Redox Signaling by Dietary Polyphenols," *Biochemical Pharmacology*, Vol. 72, No. 11, 2006, pp. 1439-1452. [doi:10.1016/j.bcp.2006.07.004](https://doi.org/10.1016/j.bcp.2006.07.004)
- [20] N. Tirkey, G. Kaur, G. Vij and K. Chopra, "Curcumin, a Diferuloylmethane, Attenuates Cyclosporine-Induced Renal Dysfunction and Oxidative Stress in Rat Kidneys," *BMC Pharmacology*, Vol. 5, 2005, pp. 1-15. [doi:10.1186/1471-2210-5-15](https://doi.org/10.1186/1471-2210-5-15)
- [21] N. Khanna, "Turmeric: Nature's Precious Gift," *Current Science*, Vol. 76, No. 10, 1999, pp. 1351-1356. [doi:10.1007/s10495-006-6715-5](https://doi.org/10.1007/s10495-006-6715-5)
- [22] J. Chen, X. Q. Tang, J. L. Zhi, Y. Cui, H. M. Yu, E. H. Tang, S. N. Sun, J. Q. Feng and P. X. Chen, "Curcumin Protects PC12 Cells against 1-Methyl-4-phenylpyridinium Ion-Induced Apoptosis by bcl-2-Mitochondria-ROS-iNOS Pathway," *Apoptosis*, Vol. 11, No. 6, 2006, pp. 943-953.
- [23] L. Perez-Arriaga, M. L. Mendoza-Magana, R. Cortes-Zarate, A. Corona-Rivera, L. Bobadilla-Morales, R. Troyo-Sanroman and M. A. Ramirez-Herrera, "Cytotoxic Effect of Curcumin on Giardia Lamblia Trophozoites," *Acta Tropica*, Vol. 98, No. 2, 2006, pp. 152-161.
- [24] R. S. Ramsewak, D. L. DeWitt and M. G. Nair, "Cyto-

- toxicity, Antioxidant and Anti-Inflammatory Activities of Curcumins IIII from *Curcuma Longa*,” *Phytomedicine*, Vol. 7, No. 4, 2000, pp. 303-308.
[doi:10.1016/S0944-7113\(00\)80048-3](https://doi.org/10.1016/S0944-7113(00)80048-3)
- [25] B. B. Aggarwal, C. Sundaram, N. Malani and H. Ichikawa, “Curcumin: The Indian Solid Gold,” *Advances in Experimental Medical Biology*, Vol. 595, 2007, pp. 1-75.
[doi:10.1007/978-0-387-46401-5_1](https://doi.org/10.1007/978-0-387-46401-5_1)
- [26] O. Ciftci, S. Tanyildizi and A. Godekmerdan, “Protective Effect of Curcumin on Immune System and Body Weight Gain on Rats Intoxicated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD),” *Immunopharmacology and Immunotoxicology*, Vol. 32, No. 1, 2010, pp. 99-104.
[doi:10.3109/08923970903164318](https://doi.org/10.3109/08923970903164318)
- [27] G. M. Cole, B. Teter and S. A. Frautschy, “Neuroprotective Effects of Curcumin,” *Advances in Experimental Medicine and Biology*, Vol. 595, 2007, pp. 197-212.
[doi:10.1007/978-0-387-46401-5_8](https://doi.org/10.1007/978-0-387-46401-5_8)
- [28] A. Kuhad, S. Pilkhwai, S. Sharma, N. Tirkey and K. Chopra, “Effect of Curcumin on Inflammation and Oxidative Stress in Displatin-Induced Experimental Nephrotoxicity,” *Journal of Agricultural and Food Chemistry*, Vol. 55, No. 25, 2007, pp. 10150-10155.
[doi:10.1021/jf0723965](https://doi.org/10.1021/jf0723965)
- [29] O. Ciftci, I. Ozdemir, S. Tanyildizi, S. Yildiz and H. Oguzturk, “Antioxidative Effects of Curcumin, β -Myrcene and 1,8-Cineole against 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Induced Oxidative Stress in Rats Liver,” *Toxicology and Industrial Health*, Vol. 27, No. 5, 2011, pp. 447-453.
[doi:10.1177/0748233710388452](https://doi.org/10.1177/0748233710388452)
- [30] O. Ciftci, A. Beytur, O. Cakir, N. Gurbuz and N. Vardi, “Comparison of Reproductive Toxicity Caused by Cisplatin and Novel Platinum-N-Heterocyclic Carbene Complex in Male Rats,” *Basic and Clinical Pharmacology and Toxicology*, Vol. 109, No. 5, 2011, pp. 328-333.
[doi:10.1111/j.1742-7843.2011.00737.x](https://doi.org/10.1111/j.1742-7843.2011.00737.x)
- [31] O. Ciftci, M. Aydin, I. Ozdemir and N. Vardi, “Quercetin Prevents 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Induced Testicular Damage in Rats,” *Andrologia*, Vol. 44, No. 3, 2012, pp. 164-173.
[doi:10.1111/j.1439-0272.2010.01126.x](https://doi.org/10.1111/j.1439-0272.2010.01126.x)
- [32] O. Ciftci, I. Ozdemir, M. Aydin and A. Beytur, “Beneficial Effects of Chrysin on the Reproductive System of Adult Male Rats,” *Andrologia*, Vol. 44, No. 3, 2012, pp. 181-186.
[doi:10.1111/j.1439-0272.2010.01127.x](https://doi.org/10.1111/j.1439-0272.2010.01127.x)
- [33] H. Ahsan, N. Parveen, N. U. Khan and S. M. Hadi, “Pro-Oxidant, Anti-Oxidant and Cleavage Activities on DNA of Curcumin and Its Derivatives Demethoxycurcumin and Bisdemethoxycurcumin,” *Chemical and Biological Interaction*, Vol. 121, No. 2, 1999, pp. 161-175.
[doi:10.1016/S0009-2797\(99\)00096-4](https://doi.org/10.1016/S0009-2797(99)00096-4)
- [34] G. K. Jayaprakasha, L. J. Rao and K. K. Sakariah, “Antioxidant Activities of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin,” *Food Chemistry*, Vol. 98, No. 4, 2006, pp. 720-724.
[doi:10.1016/j.foodchem.2005.06.037](https://doi.org/10.1016/j.foodchem.2005.06.037)
- [35] P. M. Wall and G. Messier, “Methodological and Conceptual Issues in the Use of Elevated Plus-Maze as a Psychological Measurement Instrument of Animal Anxiety-Like Behavior,” *Neuroscience and Biobehavior Review*, Vol. 25, No. 3, 2001, pp. 275-286.
[doi:10.1016/S0149-7634\(01\)00013-6](https://doi.org/10.1016/S0149-7634(01)00013-6)
- [36] O. E. Patrick, M. Hirohisa, K. Masahira and M. Koreaki, “Central Nervous System Bioaminergic Responses to Mechanic Trauma,” *Surgical Neurology*, Vol. 35, No. 4, 1991, pp. 273-279.
[doi:10.1016/0090-3019\(91\)90004-S](https://doi.org/10.1016/0090-3019(91)90004-S)
- [37] H. Ohkawa, N. Ohishi and K. Tgi, “Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction,” *Annals of Chemistry*, Vol. 95, No. 2, 1979, pp. 351-358.
- [38] M. J. Mangino, M. K. Murphy and G. G. Glabar, “Protective Effects of Glycine during Hypothermic Renal Ischemic Reperfusion Injury,” *American Journal of Physiology*, Vol. 261, No. 5, 1991, pp. F841-F848.
- [39] T. L. Sorell and J. H. Graziano, “Effect of Oral Cadmium Exposure during Pregnancy on Maternal and Fetal Zinc Metabolism in the Rat,” *Toxicology and Applied Pharmacology*, Vol. 102, No. 3, 1990, pp. 537-545.
[doi:10.1016/0041-008X\(90\)90048-Y](https://doi.org/10.1016/0041-008X(90)90048-Y)
- [40] C. Claverie, R. Corbella, D. Martin and C. Diaz, “Protective Effects of Zinc on Cadmium Toxicity in Rodents,” *Biological Trace Element Research*, Vol. 75, No. 1-3, 2000, pp. 1-9.
[doi:10.1385/BTER:75:1-3:1](https://doi.org/10.1385/BTER:75:1-3:1)
- [41] B. Baranski, “Effect of Exposure of Pregnant Rats to Admium on Prenatal and Postnatal Development of the Young,” *Journal of Hygiene, Epidemiology and Microbiology*, Vol. 29, No. 3, 1984, pp. 253-262.
- [42] I. Desi, L. Nagymajtenyi and H. Schulz, “Behavioural and Neurotoxicological Changes Caused by Cadmium Treatment of Rats during Development,” *Journal of Applied Toxicology*, Vol. 18, No. 1, 1998, pp. 63-70.
[doi:10.1002/\(SICI\)1099-1263\(199801/02\)18:1<63::AID-JAT475>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1099-1263(199801/02)18:1<63::AID-JAT475>3.0.CO;2-Z)
- [43] G. Liu and J. Elsner, “Review of the Multiple Chemical Exposure Factors Which May Disturb Human Behavioral Development,” *Sozial-und Praventionmedizin*, Vol. 40, 1995, pp. 209-217.
[doi:10.1007/BF01354475](https://doi.org/10.1007/BF01354475)
- [44] L. Nagymajtenyi, H. Schulz and I. Desi, “Behavioural and Functional Neurotoxicological Change Caused by Cadmium in a Three-Generational Study in Rats,” *Human and Experimental Toxicology*, Vol. 16, No. 12, 1997, pp. 691-699.
[doi:10.1177/096032719701601201](https://doi.org/10.1177/096032719701601201)
- [45] K. Lehotzky, G. Ungvary, D. Polinak and A. Kiss, “Behavioral Deficits Due to Prenatal Exposure to Cadmium Chloride in CFY Rat Pups,” *Neurotoxicology and Teratology*, Vol. 12, No. 2, 1990, pp. 169-172.
[doi:10.1016/0892-0362\(90\)90130-5](https://doi.org/10.1016/0892-0362(90)90130-5)
- [46] S. Morita, “Defense Mechanisms against Cadmium Toxicity I. A Biochemical and Histological Study of the Effects of Pretreatment with Cadmium on the Acute Oral Toxicity of Cadmium in Mice,” *Japanese Journal of Pharmacology*, Vol. 35, 1984, pp. 129-141.
[doi:10.1254/jjp.35.129](https://doi.org/10.1254/jjp.35.129)
- [47] M. Devi, D. A. Thomas, J. T. Barber and M. A. Finger-man, “Accumulation and Physiological and Biochemical Effects of Cadmium in a Simple Aquatic Food Chain,” *Ecotoxicology and Environmental Safety*, Vol. 33, No. 1, 1996, pp. 38-43.
[doi:10.1006/eesa.1996.0004](https://doi.org/10.1006/eesa.1996.0004)

- [48] B. Ognjanovic, R. V. Zikic, A. Stajn, Z. S. Saicic, M. M. Kostic and V. M. Petrovic, "The Effects of Selenium on the Antioxidant Defense System in the Liver of Rats Exposed to Cadmium," *Physiological Research*, Vol. 44, No. 5, 1995, pp. 293-300.
- [49] E. Casalino, C. Sblano and C. Landriscina, "Enzyme Activity Alteration by Cadmium Administration to Rats: The Possibility of Iron Involvement in Lipid Peroxidation," *Archives of Biochemistry and Biophysics*, Vol. 346, No. 2, 1997, pp. 171-179. [doi:10.1006/abbi.1997.0197](https://doi.org/10.1006/abbi.1997.0197)
- [50] A. Stajn, R. V. Zikic, B. Ognjanovic, Z. S. Saicic, S. Z. Pavlovic, M. M. Kostic and V. M. Petrovic, "Effect of Cadmium and Selenium on the Antioxidant Defense System in Rat Kidneys," *Comparative Biochemistry and Physiology*, Vol. 117C, No. 2, 1997, pp. 167-172.
- [51] M. M. Kostic, B. Ognjanovic, S. Dimitrijevic, R. V. Zikic, A. Stajn and G. L. Rosic, "Cadmium-Induced Changes of Antioxidant and Metabolic Status in Red Blood Cells of Rats: *In Vivo* Effects," *European Journal of Haematology*, Vol. 51, No. 2, 1993, pp. 86-92. [doi:10.1111/j.1600-0609.1993.tb01598.x](https://doi.org/10.1111/j.1600-0609.1993.tb01598.x)
- [52] R. V. Zikic, A. S. Stajn, B. I. Ognjanovic, Z. S. Saicic, M. M. Kostic, S. Z. Pavlovic and V. M. Petrovic, "The Effect of Cadmium and Selenium on the Antioxidant Enzyme Activities in Rat Heart," *Journal of Environmental Pathology, Toxicology and Oncology*, Vol. 17, No. 3-4, 1998, pp. 259-264.
- [53] S. Z. Pavlovic, B. I. Ognjanovic, A. S. Stajn, R. V. Zikic, Z. S. Saicic and V. M. Petrovic, "Antioxidant Defense System in Skeletal Muscle of Rats Treated with Cadmium. A Possible Protective Role of Coenzyme Q10," *Jugoslavia Medical Biochemistry*, Vol. 20, No. 4, 2001, pp. 229-235.
- [54] E. Casalino, C. Sblano and C. Landriscina, "Enzyme Activity Alteration by Cadmium Administration to Rats: The Possibility of Iron Involvement in Lipid Peroxidation," *Archives of Biochemistry and Biophysics*, Vol. 346, No. 2, 1997, pp. 171-179. [doi:10.1006/abbi.1997.0197](https://doi.org/10.1006/abbi.1997.0197)
- [55] J. G. Wilson, "Mechanisms of Teratogenesis," *American Journal of Psychiatry*, Vol. 136, 1973, pp. 129-132.
- [56] R. M. Freitas, S. M. M. Vasconcelos, F. C. F. Souza, G. S. B. Viana and M. M. F. Fonteles, "Monoamine Levels after Pilocarpine-Induced Status Epilepticus in Hippocampus and Frontal Cortex of Wistar Rats," *Neuroscience Letters*, Vol. 370, No. 2-3, 2004, pp. 196-200. [doi:10.1016/j.neulet.2004.08.024](https://doi.org/10.1016/j.neulet.2004.08.024)
- [57] V. S. Nascimento, A. A. Oliveira, R. M. Freitas, F. C. Sousa, S. M. M. Vasconcelos, G. S. B. Viana and M. M. F. Fonteles, "Pilocarpine-Induced Status Epilepticus: Monoamine Level, Muscarinic and Dopaminergic Receptors Alterations in Striatum of Young Rats," *Neuroscience Letters*, Vol. 383, No. 1-2, 2005, pp. 165-170. [doi:10.1016/j.neulet.2005.04.006](https://doi.org/10.1016/j.neulet.2005.04.006)
- [58] V. D. Petkov, S. Belcheva, E. Konstantinova and R. Kehayov, "Participation of Different 5-HT Receptors in the Memory Process in Rats and Its Modulation by the Serotonin Depletor p-Chlorophenylalanine," *Acta Neurobiologiae Experimentalis (Wars)*, Vol. 55, 1995, pp. 243-252.
- [59] G. M. Abu-Taweel, J. S. Ajarem and M. Ahmad, "Neurobehavioral Toxic Effects of Perinatal Oral Exposure to Aluminum on the Developmental Motor Reflexes, Learning, Memory and Brain Neurotransmitters of Mice Offspring," *Pharmacology Biochemistry and Behavior*, Vol. 101, No. 1, 2012, pp. 49-56. [doi:10.1016/j.pbb.2011.11.003](https://doi.org/10.1016/j.pbb.2011.11.003)
- [60] G. Richter-Levin and M. Segal, "The Effects of Serotonin Depletion and Raphe Grafts on Hippocampal Electrophysiology and Behavior," *The Journal of Neuroscience*, Vol. 11, No. 6, 1991, pp. 1585-1596.
- [61] I. S. Jamall and J. J. Sprowls, "Effects of Cadmium and Dietary Selenium on Cytoplasmic and Mitochondrial Antioxidant Defense Systems in the Heart of Rats Fed High Dietary Copper," *Toxicology and Applied Pharmacology*, Vol. 87, No. 1, 1987, pp. 102-110. [doi:10.1016/0041-008X\(87\)90088-3](https://doi.org/10.1016/0041-008X(87)90088-3)
- [62] S. Sarkar, P. Yadav and D. Bhatnagar, "Lipid Peroxidative Damage on Cadmium Exposure and Alterations in Antioxidant System in Rat Erythrocytes: A Study with Relation to Time," *Biometals*, Vol. 11, No. 2, 1998, pp. 153-157. [doi:10.1023/A:1009286130324](https://doi.org/10.1023/A:1009286130324)
- [63] E. Casalino, G. Valzaretta, C. Sblano, V. Landriscina, M. Felice Tesse and C. Landriscina, "Antioxidant Effect of Hydroxytyrosol (DPE) and Mn²⁺ in Liver of Cadmium-Intoxicated Rats," *Comparative Biochemistry and Physiology*, Vol. C133, No. 4, 2002, pp. 625-632.
- [64] B. Ognjanovic, S. Z. Pavlovic, S. D. Maletic, R. V. Žikic, A. Stajn, R. M. Radojicic, Z. S. Saicic and V. M. Petrovic, "Protective Influence of Vitamin E on Antioxidant Defense System in the Blood of Rats Treated with Cadmium," *Physiological Research*, Vol. 52, No. 5, 2003, pp. 563-570.
- [65] Z. A. Shaikh, T. T. Vu and K. Zaman, "Oxidative Stress as a Mechanism of Chronic Cadmium-Induced Hepatotoxicity and Renal Toxicity and Protection by Antioxidants," *Toxicology and Applied Pharmacology*, Vol. 154, No. 3, 1999, pp. 256-263. [doi:10.1006/taap.1998.8586](https://doi.org/10.1006/taap.1998.8586)
- [66] M. Waisberg, P. Joseph, B. Hale and D. Beyersmann, "Molecular and Cellular Mechanisms of Cadmium Carcinogenesis: A Review," *Toxicology*, Vol. 192, No. 2-3, 2003, pp. 95-117. [doi:10.1016/S0300-483X\(03\)00305-6](https://doi.org/10.1016/S0300-483X(03)00305-6)
- [67] S. J. Stohs, D. Bagchi, E. Hassoun and M. Bagchi, "Oxidative Mechanisms in the Toxicity of Chromium and Cadmium Ions," *Journal of Environmental Pathology, Toxicology and Oncology*, Vol. 20, No. 2, 2001, pp. 77-88. [doi:10.1615/JEnvironPatholToxicolOncol.v20.i2.10](https://doi.org/10.1615/JEnvironPatholToxicolOncol.v20.i2.10)
- [68] V. Calabrese, D. A. Butterfield and A. M. Stella, "Nutritional Antioxidants and the Heme Oxygenase Pathway of Stress Tolerance: Novel Targets for Neuroprotection in Alzheimer's Disease," *Italian Journal of Biochemistry*, Vol. 52, No. 4, 2003, pp. 177-181.
- [69] K. Kitani, T. Yokozawa and T. Osawa, "Interventions in Aging and Age-Associated Pathologies by Means of Nutritional Approaches," *Annals of New York Academy of Sciences*, Vol. 1019, No. 1, 2004, pp. 424-426. [doi:10.1196/annals.1297.075](https://doi.org/10.1196/annals.1297.075)
- [70] R. Motterlini, R. Foresti, R. Bassi and C. J. Green, "Curcumin, an Antioxidant and Anti-Inflammatory Agent, In-

- duces Heme Oxygenase-1 and Protects Endothelial Cells against Oxidative Stress," *Free Radical Biology and Medicine*, Vol. 28, 2000, pp. 1303-1312.
[doi:10.1016/S0891-5849\(00\)00294-X](https://doi.org/10.1016/S0891-5849(00)00294-X)
- [71] A. Ray, "Cancer Preventive Role of Selected Dietary Factors," *Indian Journal of Cancer*, Vol. 42, No. 1, 2005, pp. 11-20. [doi:10.4103/0019-509X.15095](https://doi.org/10.4103/0019-509X.15095)
- [72] A. I. Ghoneim, A. B. Abdel-Naim, A. E. Khalifa and E. S. El-Denshary, "Protective Effects of Curcumin against Ischaemia/Reperfusion Insult in Rat Forebrain," *Pharmacological Research*, Vol. 46, No. 3, 2002, pp. 273-279.
[doi:10.1016/S1043-6618\(02\)00123-8](https://doi.org/10.1016/S1043-6618(02)00123-8)
- [73] M. Thiyagarajan and S. S. Sharma, "Neuroprotective Effect of Curcumin in Middle Cerebral Artery Occlusion Induced Focal Cerebral Ischemia in Rats," *Life Sciences*, Vol. 74, No. 8, 2004, pp. 969-985.
[doi:10.1016/j.lfs.2003.06.042](https://doi.org/10.1016/j.lfs.2003.06.042)
- [74] V. Rajakrishnan, P. Viswanathan, K. N. Rajasekharan and V. P. Menon, "Neuroprotective Role of Curcumin from Curcuma Longa on Ethanolinduced Brain Damage," *Phytotherapy Research*, Vol. 13, No. 7, 1999, pp. 571-574.
[doi:10.1002/\(SICI\)1099-1573\(199911\)13:7<571::AID-PTR494>3.0.CO;2-7](https://doi.org/10.1002/(SICI)1099-1573(199911)13:7<571::AID-PTR494>3.0.CO;2-7)
- [75] G. P. Lim, T. Chu, F. Yang, W. Beech, S. A. Frautschy and G. M. Cole, "The Curry Spice Curcumin Reduces Oxidative Damage and Amyloid Pathology in an Alzheimer Transgenic Mouse," *Journal of Neuroscience*, Vol. 21, No. 21, 2001, pp. 8370-8377.
- [76] S. J. Stohs and D. Bagchi, "Oxidative Mechanisms in the Toxicity of Metal Ions," *Free Radical Biology and Medicine*, Vol. 18, No. 2, 1995, pp. 321-336.
[doi:10.1016/0891-5849\(94\)00159-H](https://doi.org/10.1016/0891-5849(94)00159-H)
- [77] R. Anane and E. E. Creppy, "Lipid Peroxidation as Pathway of Aluminium Cytotoxicity in Human Skin Fibroblast Cultures: Prevention by Superoxide Dismutase and Catalase and Vitamins E and C," *Human Experimental Toxicology*, Vol. 20, No. 9, 2001, pp. 477-481.
[doi:10.1191/096032701682693053](https://doi.org/10.1191/096032701682693053)
- [78] P. O'brien and H. J. Salasinski, "Evidence That the Reactions of Cadmium in the Presence of Metallothionein Can Produce Hydroxyl Radicals," *Archives of Toxicology*, Vol. 72, No. 11, 1998, pp. 690-700.
[doi:10.1007/s002040050562](https://doi.org/10.1007/s002040050562)
- [79] T. Koizumi, G. Shirakura, H. Kumagai, H. Tatsumoto and K. T. Suzuki, "Mechanism of Cadmium-Induced Cytotoxicity in Rat Hepatocytes: Cadmium-Induced Active Oxygen-Related Permeability Changes of the Plasma Membrane," *Toxicology*, Vol. 114, 1996, pp. 124-134.
[doi:10.1016/S0300-483X\(96\)03477-4](https://doi.org/10.1016/S0300-483X(96)03477-4)
- [80] S. K. Tandon, S. Singh, S. Prasad, K. Khandekar, V. K. Dwivedi, M. Chatterjee and N. Mathur, "Reversal of Cadmium Induced Oxidative Stress by Chelating Agent, Antioxidant or Their Combination in Rat," *Toxicology Letters*, Vol. 145, No. 3, 2003, pp. 211-217.
[doi:10.1016/S0378-4274\(03\)00265-0](https://doi.org/10.1016/S0378-4274(03)00265-0)
- [81] B. Ognjanovic, S. D. Markovic, S. Z. Pavlovic, R. V. Žikic, A. Štajn and Z. S. Saicic, "Combined Effects of Coenzyme Q10 and Vitamin E in Cadmium Induced Alterations of Antioxidant Defense System in the Rat Heart," *Environmental Toxicology and Pharmacology*, Vol. 22, 2006, pp. 219-224. [doi:10.1016/j.etap.2006.03.008](https://doi.org/10.1016/j.etap.2006.03.008)
- [82] B. Halliwell and J. M. C. Gutteridge, "Free Radicals in Biology and Medicine," 3rd Edition, Oxford University Press, New York, 1999.
- [83] M. Mates, "Effects of Antioxidant Enzymes in the Molecular Control of Reactive Oxygen Species Toxicology," *Toxicology*, Vol. 153, No. 1, 2000, pp. 83-104.
[doi:10.1016/S0300-483X\(00\)00306-1](https://doi.org/10.1016/S0300-483X(00)00306-1)
- [84] O. W. Griffith, "Biological and Pharmacologic Regulation of Mammalian Glutathione Synthesis," *Free Radical Biology and Medicine*, Vol. 27, No. 9-10, 1999, pp. 922-935. [doi:10.1016/S0891-5849\(99\)00176-8](https://doi.org/10.1016/S0891-5849(99)00176-8)
- [85] R. E. Beyer, "The Role of Ascorbate in Antioxidant Protection of Biomolecules: Interaction with Vitamin E and Coenzyme Q," *Journal of Bioenergy and Biomembrane*, Vol. 26, No. 4, 1994, pp. 349-358.
[doi:10.1007/BF00762775](https://doi.org/10.1007/BF00762775)