

Beneficial Effects of Curcumin in Maternal and Fetal Oxidative Stress and Brain Damage Induced by Gestational Lead Administration

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This study was planned to evaluate the protective role of curcumin (Cur) against maternal and fetal oxidative stress and cerebral damage induced by lead (Pb) during pregnancy. In this study, positively pregnant female rats were divided into seven groups: control group, Curgroup (300 mg/kg of Cur/b.wt.), DMSO group (50% DMSO), two Pb-treated groups (exposed to 160 and 320 mg/kg b.wt./day of Pb acetate, respectively), and two groups treated with both Pb and Cur (exposed to Pb as previous groups together with 300 mg/kg b.wt./day of Cur). Treatments started from gestation day 1 (GD1) till day 20 (GD20) through oral gavage once a day, where the mother rats of different experimental groups were sacrificed to obtain the fetuses. Different chemical parameters were assessed. Brain specimens from the mother and fetal groups were processed with examination. The results displayed that Pb administration to pregnant rats resulted in a dose-dependent toxicity for both mothers and fetuses. Also, there was a significant rise in lipid peroxidation and decreased antioxidant enzyme activities in the brains of the different Pb-treated groups. The histological examination of the brain of treated dams and fetuses showed marked alterations. Co-treatment of Cur along with Pb caused a significant decrease in Pb levels as compared with those treated with Pb alone, improving the oxidative condition with amelioration of the brain's histopathological changes. In conclusion, co-administration of Cur could have ameliorative effect against Pb-induced neurotoxicity through the reduction of oxidative stress and reversal of histopathological changes.

Keywords: Lead; oxidative stress; brain; curcumin; fetal toxicity.

Throughout history and even now, Lead (Pb) toxicity is a chief environmental health issue, especially for pregnant women and young children. Pb has been detected in all biological systems, and, despite its usefulness in life as an ingredient

in many compounds in multiple industries, it is the most toxic heavy metals, causing various negative effects on multiple systems of the body¹. Pb can reach the body via ingestion or inhalation and is absorbed mainly in the respiratory

and gastrointestinal tracts where it is transported to different tissues².

Although Pb has a wide range of impacts on body system toxicities, its neurotoxic effect is the most prominent. It has been described that cerebrum is a vulnerable target to Pb cause it contains low levels of enzymes that are responsible for defending it against oxidative stress and because of its high myelin-related content, which increases the vulnerability to peroxidation³. Also, it has been stated that Pb can interrupt the structure of the blood-brain barrier by destructing endothelial and glial cells and disturbing the building of tight junctions between barrier cells⁴.

Recently, Pb developmental toxicity has occurred as a major health problem for pregnant cause the fetus is vulnerable to Pb's many toxic agents⁵. Gestational Pb exposure produces toxic effects, that include teratogenesis, risk of low birth weight and reduced mental development⁶. Moreover, studies have proved that developmental Pb exposure exerts neurotoxicity during differentiation, synaptogenesis, and end-stages of brain development⁷.

Accumulating data supporting the role of oxidative stress in the pathophysiology of Pb toxicity has recently been reported. Pb can induce oxidative damage to cellular components involved in the production of reactive oxygen species (ROS). The mechanisms for Pb-induced oxidative stress may be either direct or indirect by uprising the creation of lipid peroxidation to levels that can disturb cell membrane functions, lipid metabolism and the antioxidant defense systems of cells^{8,9}. In addition, interruption of the prooxidant/antioxidant balance via excessive production of ROS have a significant role in brain pathology¹⁰. The effect of Pb on fetal growth has caused great concern as many studies have reported shortfalls in prenatal development in cases where mothers were exposed to high Pb levels during their pregnancies. Also, it is worth mentioning that Pb exposure during early childhood has been described to cause various toxic effects in both humans and animals¹¹. The effects of Pb on pregnancy and the fetus are complex and not completely understood in cases where both the mother and her fetus are victims of high Pb exposure. It has been proven that Pb can cross the placenta, and there is a positive correlation between

Pb levels in both maternal and umbilical cord blood¹².

In the last few years, greater attention has been given to the curative potentials of medicinal plants as natural antioxidants, due to their lack of side effects and low cost. Also, many clinical studies have suggested that the antioxidants in fruits and vegetables are key in minimizing many chronic diseases incidence¹³. One of these plants is curcumin (Cur)¹⁴, which is a famous, biologically active phenolic compound that is a chief element in turmeric, a yellow spice that is removed from the rhizome of *Curcuma longa* L. (family Zingiberaceae)^{15,16}. Cur exhibits limited solubility in water and good solubility in chloroform and dimethyl sulfoxide (DMSO), which may be account for its low bioavailability¹⁷.

Cur is reported to have many therapeutic features, including anti-inflammatory¹⁸, hypoglycemic¹⁹, hypocholesterolemic²⁰, chelating²¹, and antioxidant²²⁻²⁴. The antioxidant property of Cur is due its effective scavenging of ROS. It also decreases the level of lipid peroxides and augments the activity of antioxidant enzymes²⁵. Some studies have reported that Cur could protect against different brain disorders and neurodegeneration through suppression of oxidative stress²⁶⁻²⁸.

Cur has been used for a long time as a traditional medication during pregnancy, and no medical authority recommends against its use²⁹. It is beneficial in treating several disorders that occur commonly in pregnant women, such as anorexia, coryza, cough, and sinusitis^{26-28,30}. Cur is highly lipophilic and is capable of crossing the blood-brain barrier, as confirmed by its high concentration in the fetal brain after oral intake³¹. The current study was intended to investigate the protective role of Cur against maternal and fetal developmental parameters, oxidative stress, and cerebral damage, which was produced by two doses of Pb during pregnancy.

MATERIAL AND METHODS

Chemicals

Lead acetate (PbAc) pure crystals with a molecular weight of 379.33 and analytical-grade were purchased from Sigma Chemical Company (USA), and other chemicals and staining reagents

used in this study were purchased through local scientific agents in Jeddah, Saudi Arabia.

Animals and experimental work

Adult, sexually mature nulliparous female Sprague-Dawley albino rats (weighing 200-225 grams at the beginning of the experiment) were used in this study. They were obtained from the Animal House of King Fahd Medical Research Center. All experimental procedures were carried out in accordance with the international guidelines for the care and use of animals in the laboratory. This study was approved and registered by the Committee of Animal Investigations, Faculty of Medicine, King Abdulaziz University. During the study, the female rats were kept in separate metallic cages under standard temperature ($24 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$), and lighting (12h:12h light/dark) conditions. They were fed a standard chow diet *ad libitum* and had free access to water. After acclimatization for two weeks, mating procedure was conducted by placing the individual females overnight in the home cage of a singly-housed male of the same stock. Gestation was confirmed by positive identification of spermatozoa in a vaginal lavage smear; the day of confirmation is designated as gestation day 0 (GD0), with subsequent days of gestation numbered accordingly³². Only positively pregnant females were chosen and randomly divided into the following seven groups (eight-rats each):

- Group I: the female rats were treated with deionized water only from GD 1-20 of pregnancy (control group) through oral gavage.
- Group II (Cur): the female rats were treated with Cur at doses of 300 mg/kg b.wt. through oral gavage once a day from GD 1-20 of pregnancy.
- Group III (DMSO): the female rats were treated with 50% DMSO (solvent of Cur) through oral gavage once a day from GD 1-20³³⁻³⁵.
- Group IV (L160): the female rats were given 160 ppm of Pb acetate (PbAc) through oral gavage once a day from GD 1-20.
- Group V (L160 + Cur): the female rats were given 160 ppm of PbAc plus Cur (300 mg/kg b.wt.) by oral gavage once a day from GD 1-20.
- Group VI (L320): the female rats were given 320 ppm of PbAc by oral gavage from GD 1-20.
- Group VII (L320 + Cur): the female rats

were given 320 ppm of PbAc plus garlic extract (300 mg/kg b.wt.) by oral gavage once a day from GD 1-20.

Pb solution was prepared by dissolving PbAc in acidified, distilled water in concentrations of 0.1 and 0.2% (w/v), containing 160 and 320 ppm of Pb respectively³⁶. Cur solution was prepared by dissolving the powder in 50% DMSO to give a dose of 300 mg/kg body weight and diluted further with distilled water in 1.0 ml volume; this was administered orally once a day³⁷.

Evaluations of pregnant rats and fetuses

The pregnant female rats of each group were observed daily throughout gestation for mortality, morbidity, and body-weight gain following treatment. On day 20 of gestation and under ether anesthesia, blood samples were taken via cardiac puncture and centrifuged at 3,000 rpm for 15 minutes to separate the sera, which were stored at -80°C . Then, the abdominal cavity was opened, the gravid uterine horns were removed and weighed, and the uterine contents were examined to determine the number of corpora lutea, implantation sites, and resorptions (embryonic death); also, the number and position of viable and dead fetuses were counted. Additionally, pre-implantation losses were calculated as: $(\text{number of corpora lutea} - \text{number of implantations}) \times 100 / \text{number of corpora lutea}$. Then, all viable fetuses and placentas were removed and weighed separately. Following this, fetal crown-rump length, head length, and biparietal diameter were measured using a Vernier caliper and recorded. The heads of the mother and fetal rats of each group were opened, and the cerebellum of mother and fetal brains were quickly extracted and weighed. All extracted specimens were divided sagittally into two halves; the right halves were fixed in 10% neutral-buffered formalin for histological and immunohistochemical study, and the left halves were frozen and stored at -70°C .

Measurement of Pb levels in the brain tissues

Left halves of the brains from rat mothers and fetuses of all groups were digested in concentrated HNO_3 Suprapur in a shaking water bath at 60°C for 30 minutes. After digestion, the solution was diluted (1:5 v/v) with deionized water. Pb levels were measured using a graphite furnace atomic absorption spectrophotometer (Perkin-Elmer Model 3030) at King Fahd Medical Research

Center, King Abdulaziz University. Results were expressed as μg of Pb/dl blood, and Pb levels in brain and placenta were expressed as $\mu\text{g/g}$ tissue weight^{36, 38, 39}.

Biochemical assays

Left halves of the brains from rat mothers and fetuses of all groups were homogenized (10% w/v) in ice-cold 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged twice at 4,000 rpm for 15-20 minutes at 4°C. The resultant supernatant was used for estimation of various biochemical assays. The lipid peroxidation (LPO) was estimated by determining the amount of malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids using a thiobarbituric acid-reactive substances assay kit (BioAssay Systems, Hayward, CA)⁴⁰. The antioxidant enzyme activity of the rat brains was evaluated by determining the superoxide dismutase (SOD) activity. This was measured using a SOD assay kit (Cayman), in which a tetrazolium salt was used for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine⁴¹. Catalase (CAT) activity was assayed according to the peroxidatic function of catalase using a catalase assay kit (Cayman)⁴¹. Glutathione peroxidase (GPx) activity was assayed by coupling the enzyme procedure with glutathione reductase using a GPx assay kit (Cayman)⁴¹.

Histological and immunohistochemical methods

The fixed right halves of the maternal and fetal brains of all experimental groups were dehydrated, cleared, and embedded in paraffin, and then sections of 5 μm thickness were cut. Some sections were stained with hematoxylin-eosin (H&E) for general examination. Other sections were immunohistochemically stained with a modified avidin-biotin peroxidase technique for anti-GFAP to examine the astrocytes. Deparaffinized and rehydrated sections were treated with 0.01 M citrate buffer (pH 6.0) for 10 minutes to unmask antigens, and then incubated with a monoclonal antibody against GFAP (1:100 monoclonal mouse anti-GFAP) for 18-20 hours. Detection of the antibody was carried out using a biotin-streptavidin detection system with 0.05% diaminobenzidine as a chromogen and counterstained with hematoxylin. GFAP-positive cells appeared brown and their nuclei appeared blue. The positive results were indicated by brown coloration of the cell membrane

and cytoplasm of the astrocytes. Finally, all slides were examined with an Olympus BX53 microscope equipped with a camera (Olympus, Tokyo, Japan) at different magnifications⁴².

Quantitative Morphometric study

Ten slides of non-overlapping fields from each group (at least five animals/group), at least one slide from each animal, were analyzed with the use of Image-Pro Plus v6 (Media Cybernetics Inc., Bethesda, Maryland, USA) and Image J (National Institute of Health, USA) was used to quantify cells, measure area% covered by cells and colour intensity (GFAP immunoreaction in astrocytes) within the cerebral cortex. All morphometric measurements were done at $\times 100$ magnification^{42, 43}. For each calculation, the background was determined by manually adjusting the density window of the system until only the GFAP-positive tissue was selected. i.e. Digital brightfield images were uploaded into the image J software and scale set using a digital micrometer gauge reading to convert measurements in pixels to microns, and this was applied to all images. Cells were counted using the cell-counter plug-in available on the image J software after a grid had been applied across the image; number of different cell types in the respective brain regions was then counted. Total number of cells, area% covered by cells and average cell size were calculated using the automated cell-counter after thresholding for colour. Then, immunoreactive blood vessels, nonspecific staining, and artifacts were omitted before quantitation. When a subfield was too large to be analyzed at one time, several measurements of non-overlapping areas were made and then averaged. For whole cortex, the GFAP-positive area was divided by the total area analyzed to obtain an estimate of the area% occupied by astrocytes and their processes⁴³⁻⁴⁵.

Statistical analysis

All the data were presented as mean \pm standard deviation of studied parameters from each group. Data were analyzed using a one-way analysis of variance followed by Bonferroni's *post hoc* test or Student's t-test, wherever applicable. Four different maternal and fetal parameters were used. All statistical analyses were done using the Statistical Package for the Social Sciences (SPSS, version 23) (USA). The values of $P < 0.05$ were considered significant.

RESULTS

Maternal findings

During the experiment, two pregnant rats from the high-dose Pb-treated group died. Post-mortem examination revealed that the fetuses aborted or died, and the treatment had no effect on the duration of the pregnancy. As represented in Table 1, there was a significant reduction in both maternal weight gain and placental and brain weights in Pb-treated groups, which was more marked in the group that received the larger Pb dose, when compared to the control. Also, it was noticed that the co-administration of Cur improved these effects.

Fetal findings

As seen in Table 2, the Pb-treated groups displayed a reduction of fetal weight and other fetal growth parameters (crown-rump length, head length, biparietal diameter, umbilical cord length), which was more marked in the group that received the larger Pb dose, when compared to the control; the co-administration of Cur along with Pb improved these effects.

Materno-fetal Pb analysis

The data in Table 3 show that there were parallel and significant increases in the mean values of Pb-level concentrations in the maternal blood and brains and fetal brains in Pb-treated groups than in the control group; this was higher in the group that received the larger Pb dose. Also, co-administration of Cur along with Pb resulted in the reduction of Pb levels versus the control group. Analysis showed that Pb content in the fetal brain was positively correlated to maternal Pb levels, which indicates Pb transfer from the mother to the fetus.

Biochemical assays

As seen in Table 4, there was a significant increase of MDA levels in the maternal brains of Pb-treated groups, which was significant when compared to the control group; this was higher in the group that received the larger Pb dose. Moreover, there was a decrease of SOD, CAT, and GPx levels in Pb-treated groups in a dose-dependent manner, which was significant when compared to the control group. Co-administration of Cur along with Pb resulted in the decrease of

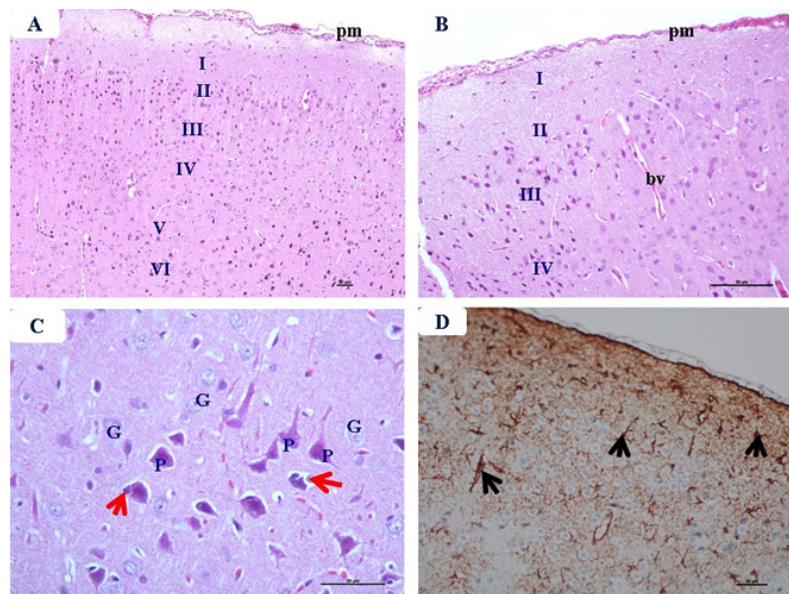


Fig. 1. Photomicrographs of cerebral cortex from control and Cur treated mother rats showing A & B) the normal layering pattern in the form of six layers; I: outer molecular layer, II: external granular layer, III: external pyramidal layer, IV: inner granular layer, V: inner pyramidal layer, VI: polymorphic layer. C) the pyramidal cells (P) with their multipolar shape, vesicular nuclei and basophilic cytoplasm. Granular cells (G) were seen with large nuclei, prominent nucleolus and little cytoplasm. The smaller neuroglia cells are scattered (\uparrow). D) a few GFAP positive astrocytes with brown cytoplasmic granules (\uparrow). pm= pia matter, bv= blood vessel. [A, B & C: H & E X 100, 200 & 400, D: GFAP X 200]

MDA level and the increase of antioxidant enzyme activities.

Regarding the fetal brains, Table 5 illustrates that there was a significant increase

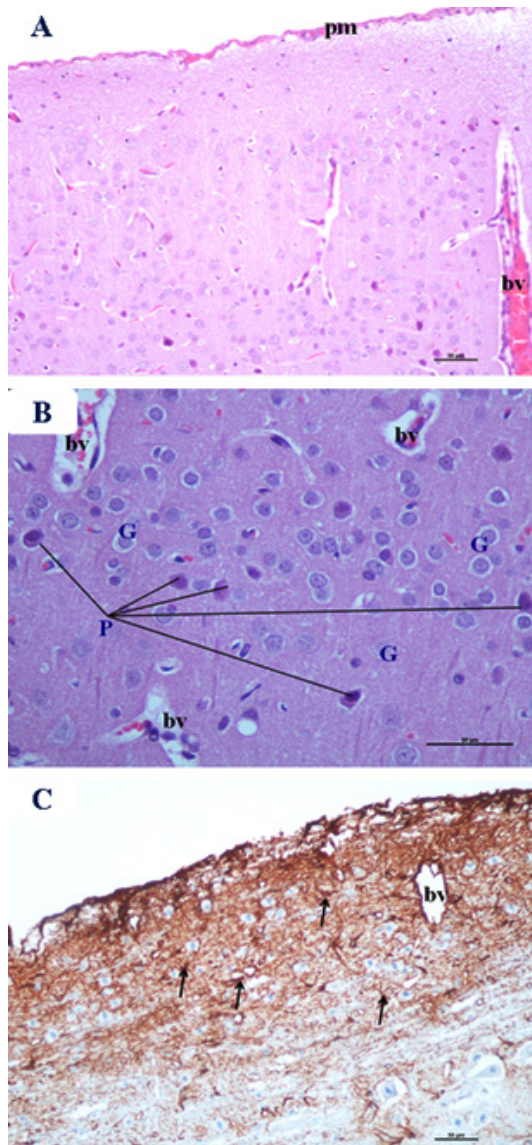


Fig. 2. Photomicrographs of cerebral cortex from low dose Pb-treated mother rats showing A) a little disorganization of cortical layers with some congested blood vessels (bv). B) some shrunken pyramidal cells (P) with loss of their processes, dark cytoplasm and small darkly stained nucleus. Some granular cells (G) are surrounded with halos. C) an increase in the GFAP immunoreactive astrocytes (↑) as compared to the control group. pm= pia matter [A&B: H&E X100 & 400, C: GFAP X 200]

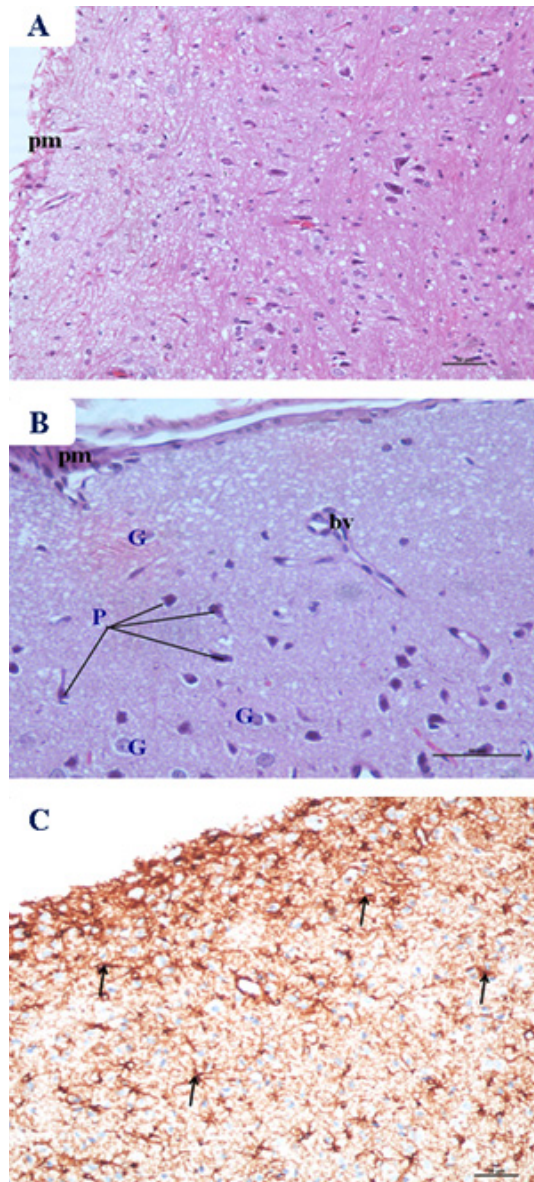


Fig. 3. Photomicrographs of cerebral cortex from high dose Pb-treated mother rats showing A) a distortion and disappearance of normal arranged cortical layers with dilated blood vessels. B) some pyramidal cells (P) were appeared darkly stained and irregular in shape with pyknotic nuclei; other cells showed marked vacuolization with faintly stained cytoplasm. Notice that some granular cells are shrunken and deeply stained (G). C) a marked increase in the number of GFAP immunoreactive astrocytes (↑) as compared to low dose Pb treated and control groups. pm= pia matter, bv= blood vessel. [A&B: H&E X100 & 400, C: GFAP X 200]

of MDA levels in the fetal brains of Pb-treated groups, which was significant when compared to the control group; this was higher in the group that received the larger Pb dose. Also, there was a decrease of SOD, CAT, and GPx levels in Pb-treated groups, which was significant when compared to the control group; these levels were lower in the group given the higher Pb dose. In contrast, co-administration of Cur along with Pb resulted again in the decrease of MDA and the increase of antioxidant enzyme activities.

Histological results

Examination of the H&E-stained sections of the cerebral cortex of the mother rats showed that there were nearly the same findings in the control, Cur, and DMSO-treated mother groups. In general, normal histoarchitecture was seen in the six layers of the cerebral cortex (Fig. 1 A, B & C). In the Pb-treated groups, the examination revealed variable degrees of histopathological changes, which were less evident in the lower dose groups (Fig. 2 A & B) than in the higher dose groups (Fig. 3 A & B). Disorganization and lack of characteristic, typical, layered structures were

seen with some vacuoles of variable sizes between cells. Some pyramidal cells appeared irregular in shape and darkly stained with loss of their processes; others showed marked vacuolization with faintly stained cytoplasm. Most of granular cells were affected and became ill-defined and faintly stained with pericellular halos. Also, some apoptotic cells with dense nuclei were observed. Regarding the Pb and Cur treated groups (Fig. 4 A, B & C), the examination showed improvement of histological appearance of the cortical layers, which appeared similar to the control group with decreased cellular damage, especially with the low Pb-dose group. The pyramidal cells appeared almost normal with vesicular nuclei; a few were still vacuolated with faintly stained acidophilic cytoplasm. The immunohistochemical examination of GFAP-stained sections of the cerebral cortex from the above groups revealed a few GFAP-positive immuno-reactive astrocytes with their processes dispersed in between different cell layers of the cerebral cortex in the control, Cur, and DMSO-treated groups (Fig. 1 D). In the Pb-treated groups, an apparent increase in the number

Table 1. Effect of gestational Pb and Cur co-administration on pregnant rats' parameters (Mean \pm SD)

Groups	Maternal Wt. Gain (gm)	Placental Wt. (gm)	Brain Wt. (gm)
Control (n=8)	96.6 \pm 11.9	0.73 \pm 0.011	1.74 \pm 0.09
Cur (n=8)	100.1 \pm 9.6	0.74 \pm 0.031	1.75 \pm 0.11
DMSO (n=8)	98.35 \pm 10.75	0.735 \pm 0.021	1.745 \pm 0.10
L160 (n=8)	80.6 \pm 12.9	0.63 \pm 0.014	1.59 \pm 0.07
	P ¹ <0.05	P ¹ <0.001	P ¹ <0.05
	P ² <0.01	P ² <0.001	P ² <0.01
	P ³ <0.05	P ³ <0.001	P ³ <0.05
L160 + Cur (n=8)	95.7 \pm 7.83	0.68 \pm 0.019	1.729 \pm 0.04
	P ⁴ <0.05	P ⁴ <0.001	P ⁴ <0.05
L320 (n=6)	75.0 \pm 6.93	0.58 \pm 0.018	1.56 \pm 0.12
	P ¹ <0.001	P ¹ <0.001	P ¹ <0.01
	P ² <0.001	P ² <0.001	P ² <0.001
	P ³ <0.001	P ³ <0.001	P ³ <0.01
L320 + Cur (n=8)	90.1 \pm 7.12	0.64 \pm 0.028	1.699 \pm 0.05
	P ⁵ <0.05	P ⁵ <0.001	P ⁵ <0.05
F value	7.668	67.733	6.545

Number of rat per treatment group = 8 except for the high lead dose. ANOVA test with Bonferroni post hoc test:

P¹: compared to control group. P²: compared to Cur group. P³: compared to DMSO group. P⁴: compared to L160 group. P⁵: compared to L320 group.

Table 2. Effect of gestational Pb and Cur co-administration on fetal and brain weights and growth parameters (Mean \pm SD)

Groups (N of each)	Fetal Wt. (gm)	Crown- Rump Length (cm)	Head Length (cm)	Biparietal Diameter (cm)	Umbilical cord length (cm)	Fetal brain Wt. (gm)
Control (n=102)	2.79 \pm 0.037	2.96 \pm 0.025	1.33 \pm 0.003	0.75 \pm 0.002	2.27 \pm 0.055	0.142 \pm 0.022
Cur (n=98)	2.83 \pm 0.043	2.95 \pm 0.015	1.34 \pm 0.012	0.76 \pm 0.011	2.25 \pm 0.045	0.145 \pm 0.027
DMSO (n=102)	2.81 \pm 0.040	2.95 \pm 0.02	1.35 \pm 0.0075	0.741 \pm 0.015	2.26 \pm 0.050	0.1435 \pm 0.0245
L160 (n=83)	2.41 \pm 0.055 P ¹ <0.001 P ² <0.001 P ³ <0.001	2.65 \pm 0.026 P ¹ <0.001 P ² <0.001 P ³ <0.001	1.24 \pm 0.004 P ¹ <0.001 P ² <0.001 P ³ <0.001	0.69 \pm 0.009 P ¹ <0.001 P ² <0.001 P ³ <0.001	2.09 \pm 0.051 P ¹ <0.001 P ² <0.001 P ³ <0.001	0.129 \pm 0.017 P ¹ <0.05 P ² <0.001 P ³ <0.01
L160 + Cur (n=92)	2.69 \pm 0.049 P ⁴ <0.001	2.79 \pm 0.028 P ⁴ <0.001	1.29 \pm 0.003 P ⁴ <0.001	0.73 \pm 0.003 P ⁴ <0.001	2.21 \pm 0.058 P ⁴ <0.001	0.141 \pm 0.029 P ⁴ <0.05
L320 (n=79)	2.23 \pm 0.041 P ¹ <0.001 P ² <0.001 P ³ <0.001	2.58 \pm 0.044 P ¹ <0.001 P ² <0.001 P ³ <0.001	1.19 \pm 0.005 P ¹ <0.001 P ² <0.001 P ³ <0.001	0.63 \pm 0.002 P ¹ <0.001 P ² <0.001 P ³ <0.001	1.93 \pm 0.026 P ¹ <0.001 P ² <0.001 P ³ <0.001	0.120 \pm 0.032 P ¹ <0.001 P ² <0.001 P ³ <0.001
L320 + Cur (n=88)	2.62 \pm 0.045 P ⁵ <0.001	2.69 \pm 0.041 P ⁵ <0.001	1.24 \pm 0.004 P ⁵ <0.001	0.71 \pm 0.001 P ⁵ <0.001	2.05 \pm 0.046 P ⁵ <0.001	0.135 \pm 0.026 P ⁵ <0.01
F value	2262.7	2671.2	8116.4	2549.1	657.55	10.909

ANOVA test with Bonferroni post hoc test. P¹: compared to control group. P²: compared to Cur group. P³: compared to DMSO group. P⁴: compared to L160 group. P⁵: compared to L320 group

Table 3. Effect of gestational Pb and Cur co-administration on materno-fetal Pb concentrations (Mean \pm SD)

Groups (N of each)	Maternal blood Pb level ($\mu\text{g}/\text{dl}$)	Maternal brain Pb level ($\mu\text{g}/\text{gm}$)	Groups (N of each)	Fetal brain Pb level ($\mu\text{g}/\text{gm}$)
Control (N=8)	5.3 \pm 0.99	0.06 \pm 0.004	Control (N=102)	0.010 \pm 0.003
Cur (N=8)	5.1 \pm 1.21	0.05 \pm 0.011	Cur (N=98)	0.008 \pm 0.002
DMSO (N=8)	5.2 \pm 1.1	0.055 \pm 0.0075	DMSO (N=102)	0.009 \pm 0.0025
L160 (N=8)	23.7 \pm 4.19	1.05 \pm 0.13	L160 (N=83)	0.43 \pm 0.011
	P ¹ <0.001	P ¹ <0.001		P ¹ <0.001
	P ² <0.001	P ² <0.001		P ² <0.001
	P ³ <0.001	P ³ <0.001		P ³ <0.001
L160 + Cur (N=8)	10.1 \pm 4.45	0.29 \pm 0.17	L160 + Cur (N=92)	0.06 \pm 0.009
	P ⁴ <0.001	P ⁴ <0.001		P ⁴ <0.001
L320 (N=6)	42.5 \pm 5.41	1.60 \pm 0.32	L320 (N=79)	0.85 \pm 0.17
	P ¹ <0.001	P ¹ <0.001		P ¹ <0.001
	P ² <0.001	P ² <0.001		P ² <0.001
	P ³ <0.001	P ³ <0.001		P ³ <0.001
L320 + Cur (N=8)	15.2 \pm 3.24	0.33 \pm 0.12	L320 + Cur (N=88)	0.23 \pm 0.07
	P ⁵ <0.001	P ⁵ <0.001		P ⁵ <0.001
F value	132.12	124.93	F value	1992.1

Number of rat per treatment group = 8 except for the high lead dose. ANOVA test with Bonferroni post hoc test. P¹: compared to control group. P²: compared to Cur group. P³: compared to DMSO group. P⁴: compared to L160 group. P⁵: compared to L320 group

Table 4. Effect of gestational Pb and Cur administration on the lipid peroxidation and antioxidant enzyme activities of the maternal brains (Mean \pm SD)

Groups	MDA (nmol/ gm tissue)	SOD (U/gm Tissue)	CAT ($\mu\text{mole}/\text{gm}$ Tissue)	GPx ($\mu\text{mol}/\text{gm}$ tissue)
Control (n=8)	17.43 \pm 0.10	69.11 \pm 9.21	250.1 \pm 4.1	22.3 \pm 1.1
Cur (n=8)	15.88 \pm 0.14	68.18 \pm 6.22	243.1 \pm 7.3	21.7 \pm 2.7
DMSO (n=8)	16.66 \pm 0.12	68.65 \pm 7.72	246.6 \pm 5.7	22 \pm 1.9
L160 (n=8)	29.42 \pm 0.86	51.32 \pm 3.41	217.3 \pm 6.3	17.4 \pm 2.4
	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001	P ¹ <0.01
	P ² <0.001	P ² <0.001	P ² <0.001	P ² <0.01
	P ³ <0.001	P ³ <0.001	P ³ <0.001	P ³ <0.01
L160 + Cur (n=8)	19.94 \pm 0.92	63.27 \pm 2.16	236.4 \pm 5.9	21.1 \pm 1.9
	P ⁴ <0.001	P ⁴ <0.001	P ⁴ <0.001	P ⁴ <0.001
L320 (n=6)	46.38 \pm 1.27	41.56 \pm 4.01	197.4 \pm 7.1	12.6 \pm 1.8
	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001
	P ² <0.001	P ² <0.001	P ² <0.001	P ² <0.001
	P ³ <0.001	P ³ <0.001	P ³ <0.001	P ³ <0.001
L320 + Cur (n=8)	23.74 \pm 0.96	59.42 \pm 3.17	218.1 \pm 7.2	19.8 \pm 3.3
	P ⁵ <0.001	P ⁵ <0.001	P ⁵ <0.001	P ⁵ <0.001
F value	1590	26.933	75.433	19.284

Number of rat per treatment group = 8 except for the high lead dose. ANOVA test with Bonferroni post hoc test:

P¹: compared to control group. P²: compared to Cur group. P³: compared to DMSO group. P⁴: compared to L160 group. P⁵: compared to L320 group.

of GFAP immuno-reactive astrocytes was observed as compared to the control animals, which was more rigorous in the group that received the high dose of Pb (Fig. 3 C) than that which received the low dose (Fig. 2 C). In the Pb- and Cur-treated group (Fig. 4 D), a noticeable decrease in the GFAP

immune-reactive astrocytes was seen as compared to the Pb-treated rats (Table 6).

Examination of the H&E-stained sections from the fetal brains belonging to the control, Cur, and DMSO-treated groups showed nearly the same, normal histological findings, which displayed

Table 5. Effect of gestational Pb and Cur administration on the lipid peroxidation and antioxidant enzyme activities of the fetal brains (Mean \pm SD)

Groups (N of each)	MDA (nmol/ gm tissue)	SOD (U/gm Tissue)	CAT (μ mole/ gm Tissue)	GPx (μ mol/ gm tissue)
Control (n=102)	6.33 \pm 1.9	0.63 \pm 0.18	16.6 \pm 4.1	15.2 \pm 2.8
Cur (n=98)	5.88 \pm 1.6	0.62 \pm 0.12	15.9 \pm 2.3	17.1 \pm 2.3
DMSO (n=102)	6.1 \pm 1.75	0.625 \pm 0.15	16.25 \pm 3.2	16.15 \pm 2.55
L160 (n=83)	11.12 \pm 2.1	0.51 \pm 0.19	23.1 \pm 6.3	11.4 \pm 2.6
	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001
	P ² <0.001	P ² <0.001	P ² <0.001	P ² <0.001
	P ³ <0.001	P ³ <0.001	P ³ <0.001	P ³ <0.001
L160 + Cur (n=92)	7.92 \pm 1.8	0.60 \pm 0.16	19.2 \pm 6.1	13.1 \pm 2.9
	P ⁴ <0.001	P ⁴ <0.01	P ⁴ <0.001	P ⁴ <0.001
L320 (n=79)	22.47 \pm 4.7	0.38 \pm 0.17	35.4 \pm 7.3	8.8 \pm 2.7
	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001	P ¹ <0.001
	P ² <0.001	P ² <0.001	P ² <0.001	P ² <0.001
	P ³ <0.001	P ³ <0.001	P ³ <0.001	P ³ <0.001
L320 + Cur (n=88)	9.53 \pm 1.7	0.54 \pm 0.15	22.4 \pm 6.8	10.7 \pm 3.1
	P ⁵ <0.001	P ⁵ <0.001	P ⁵ <0.001	P ⁵ <0.001
F value	514.68	27.228	141.05	115.44

ANOVA test with Bonferroni post hoc test: P¹: compared to control group. P²: compared to Cur group. P³: compared to DMSO group. P⁴: compared to L160 group. P⁵: compared to L320 group

Table 6. Area percentage of GFAP (Mean \pm SD)

Groups (N of each)	Maternal GFAP area percentage	Groups (N of each)	Fetal GFAP area percentage
Control (n=8)	0.563 \pm 0.188	Control (n=102)	0.285 \pm 0.026
Cur (n=8)	0.55 \pm 0.175	Cur (n=98)	0.275 \pm 0.086
DMSO (n=8)	0.57 \pm 0.19	DMSO (n=102)	0.280 \pm 0.095
L160 (n=8)	1.1 \pm 0.37	L160 (n=83)	0.55 \pm 0.185
	P ¹ <0.01		P ¹ <0.001
	P ² <0.01		P ² <0.001
	P ³ <0.01		P ³ <0.001
L160 + Cur (n=8)	0.602 \pm 0.209	L160 + Cur (n=92)	0.301 \pm 0.105
	P ⁴ <0.05		P ⁴ <0.001
L320 (n=6)	1.42 \pm 0.473	L320 (n=79)	0.71 \pm 0.237
	P ¹ <0.001		P ¹ <0.001
	P ² <0.001		P ² <0.001
	P ³ <0.001		P ³ <0.001
L320 + Cur (n=8)	0.600 \pm 0.2	L320 + Cur (n=88)	0.32 \pm 0.11
	P ⁵ <0.001		P ⁵ <0.001
F value	12.289	F value	147.08

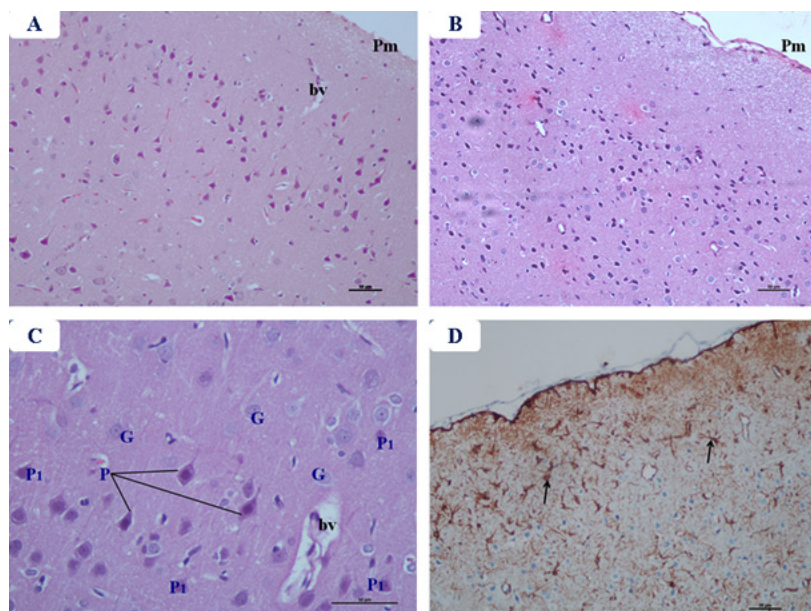


Fig. 4. Photomicrographs of cerebral cortex from Pb and Cur-treated mother rats showing: A) an improvement in the histoarchitecture with organized layers. B) more or less normal pyramidal cells (P) and granular cells (G). C) a decrease in the GFAP immunoreactive astrocytes as compared Pb-treated rats (\uparrow). pm= pia matter, bv= blood vessel. [A: H&E X100 & B: 400, C: GFAP X 200]

normal appearance of the telencephalon (which appeared with distinct structure and was formed of five zones) with its lateral ventricle (Fig. 5 A & B). In the low-dose Pb-treated group, the picture was similar with no difference from that of the control group. In the high-dose Pb-treated group (Fig. 6 A & B), the examination of fetal brains revealed variable degrees of histopathological changes where there was a less degree of maturation than of the control fetuses in the form of dilation of the lateral ventricle as well as thinning and hardly distinguishable arrangement of the telencephalic wall layers. Also, thinned ventricular and subventricular zones, widening of the intermediate zone, hypoplasia, and reduction of the cortical zone were seen. While in the fetal brains from the Pb- and Cur-treated group (Fig. 7 A & B), the examination showed regressive changes, and the picture was similar to that of the control group regarding the thickness of the telencephalic wall and size of the lateral ventricle. The immunohistochemical examination of GFAP-stained sections of the fetal brain showed negative or very little GFAP-positive cellular reaction in the superficial layers of the telencephalic wall (Fig. 5 C). In the fetal brains from the high-dose Pb-treated

group, there was apparent increase in the number of GFAP immuno-reactive astrocytes as compared to the control group (Fig. 6 C). However, in the fetal brain from Pb- and Cur-treated group, a reduction of GFAP immuno-reactive astrocytes was observed (Table 6, Fig. 7 C).

DISCUSSION

In the current study, Pb at a dose of 160 and 320 mg/kg b.wt. during pregnancy caused a significant reduction in brain weight, body weight and fetal body weight when compared to the control rats, which was obvious in those received the larger dose of Pb. In accordance with these findings, the authors reported that Pb causes several adverse health effects that are dose-dependent and somewhat irreversible⁴⁶. Also, it was found that Pb induces an important reduction in pups' body and brain weight at postnatal days 0-21 when their dams consumed drinking-water containing 300 mg/L of Pb^{12, 47}.

The central nervous system is considered to be the primary target of Pb exposure, especially in the developing brain, leading to weakening of

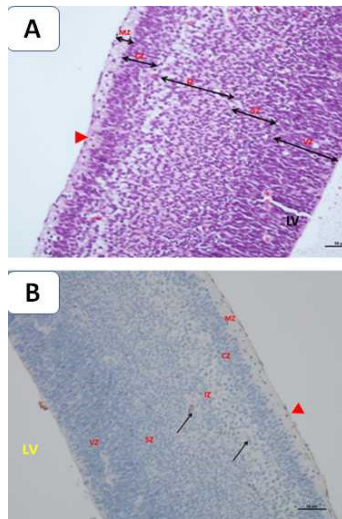


Fig. 5. Photomicrographs of the fetal brain at GD20 from control and Cur treated groups showing: A) telencephalic wall (TW), which is formed of five basic zones: marginal zone (MZ), cortical zone (CZ), intermediate zone (IZ), subventricular zone (SZ) and ventricular zone (VZ). B) negative or very few and weak GFAP positive cellular reaction (\blacktriangle), which are scattered in the superficial layers of the telencephalic wall. LV = lateral ventricle [A: H&E X 200, B: GFAP X 200].

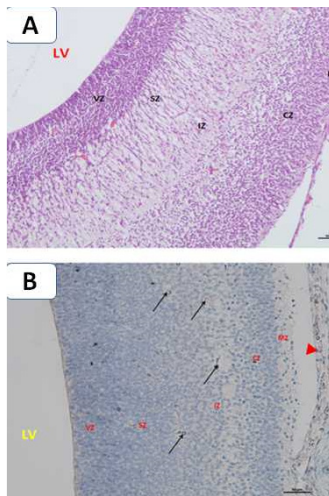


Fig. 6. Photomicrographs of the fetal brain at GD20 from high dose Pb-treated group showing A) a hardly distinguishable arrangement of the telencephalic wall layers, with thinned ventricular (VZ) and subventricular (SZ) zones and widening of the intermediate zone (IZ), hypoplasia and reduction of the cortical zone (CZ) and disruption of the marginal zone (MZ). B) a presence of GFAP positive reaction (\blacktriangle), which was more scattered in the superficial layers of the telencephalic wall. LV = lateral ventricle [A: H&E X 200, B: GFAP X 200].

cognitive abilities and interruption of behavioral development and attentiveness in children⁴⁸. These results could be explained by the fact that infants absorb 50% of Pb that are exposed to, whereas adults absorb 10%^{11, 23, 49}. Also, Pb traverses the blood-brain barrier simply while the brain is developing in a rapid growth and an intense cellular proliferation. This is due to blood-brain barrier immaturity, which caused absorption of Pb^{11, 23, 49}. Studies have revealed that the fetuses of pregnant mothers predisposed to Pb level (0.01% and 0.05% w/v orally for 45 days) exhibited higher fetal brain Pb levels⁵⁰.

The elevated maternal blood Pb level in the current results that is obvious in the group with a higher dose of Pb, shows a correlation between maternal and umbilical cord blood Pb levels. Those findings support the concept that Pb might easily cross to the placenta in a dose-responsive manner. It was described that the placenta can't protect the fetus from exposure to Pb, because it is able to cross the placental barrier freely⁵¹. Current results come in the same line with other studies that compared the Pb levels of umbilical cord blood and maternal blood and with studies that declared that the Pb in

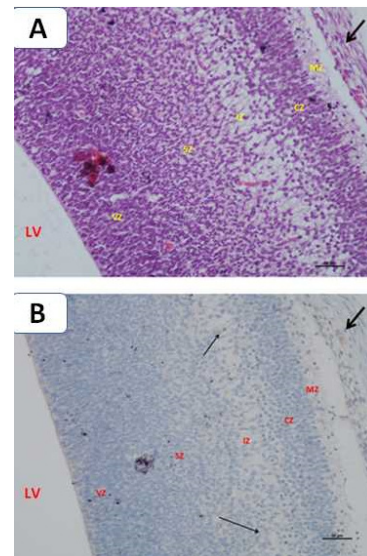


Fig. 7. Photomicrographs of the fetal brain at GD20 from high dose Pb and Cur treated group showing: A) a distinguishable arrangement of the telencephalic wall layers, which appeared similar to the control fetal brain. B) a reduction in the number of GFAP immunoreactive astrocytes (\blacktriangle). LV = lateral ventricle [A: H&E X 200, B: GFAP X 200].

new-born infants' blood was a reflection of that of the mother⁵².

Current results indicated that the dam's Pb exposure caused an increase of Pb in fetus blood and placenta, in addition to elevation of the Pb in the brain of the developing fetus. Similarly, preceding studies showed that Pb was highly concentrated in the placenta, umbilical cord and brain, and it is supposed that the placenta and umbilical cord might be respectable biomarkers of fetal Pb exposure⁵³. Furthermore, studies have demonstrated that Pb infiltrate the immature blood-brain barrier and accumulate in the developing brain, which seems to be vulnerable to metal infiltration; this results in a significant dose-dependent rise, confirming the transplacental metal passage from the mothers⁶.

In the current work, it was found that Pb causes a significant increase in LPO, as evidenced by the high MDA level in the maternal and fetal brains; this was accompanied by marked reduction of antioxidant enzymes (SOD, CAT, and GPx) in different Pb exposure groups. It was stated that Pb-induced disturbance of the prooxidant/antioxidant balance in the brain might encourage impairment to different cellular components, including nucleic acids, membrane lipids, and proteins⁵⁴. Also, it was shown that MDA level is strongly correlated with Pb concentration in the brains of exposed rats⁵⁵. Moreover, these results were consistent with earlier animal and human studies, which suggested that exposure to Pb was associated with increased oxidative stress and occurrence of neurotoxicity due to oxidative damages, as the brain consumes 20% of the body's oxygen⁵⁶.

The results also showed increased LPO in the fetal brains with pre-natal Pb exposure. It was declared that the toxic effects of Pb on the fetal brain are undoubtedly the main significant and meticulously studied consequence of intrauterine Pb exposure. This injurious effect of high Pb levels has been well-recognized^{57,58}. Such effects might be affecting the morphological developments and the sensory reflexes of the pups as well as the behavior of young adult offspring⁵⁹.

Histopathologically, the present study displayed a variable degree of structural impairment of the maternal and fetal brains in the Pb-treated groups, which were more understandable in those exposed to the higher dose of Pb. It was stated previously that Pb-induced damage occurred

primarily in the cerebrum, hippocampus and cerebellum affecting various biological activities at the molecular, cellular, and intracellular levels, which can cause morphological modifications in the brain that may persist even after Pb levels have dropped⁶⁰. In this study, there were decreased pyramidal cells along with the presence of degenerated cells with pyknotic nuclei in Pb-treated groups in comparison to control. This agreed with studies that accredited these findings to the direct effect of Pb on brain cells⁶¹. Correspondingly, Pb-induced cell death has been established in the cortex and cerebellum on neonatal rats in-vivo (2-4 weeks old). This indicates a sophisticated sensitivity in younger rats with apoptotic cells⁴⁹.

The study showed that fetal brains from Pb-exposed groups displayed reduced differentiation and cell apoptosis degeneration. Accordingly, it was noticed that the intrauterine exposure to Pb is harmfully disturbs cellular proliferation, differentiation and synaptic growth of the brain, which causes mental retardation or movement disorders⁶². Accordingly, studies have described that Pb exposure during embryonic development causes cortical and cerebellar impairment in newborn and in the developing hippocampus. These effects happen cause of Pb interferes with several proliferative and apoptotic pathways⁶³.

In the current study, the effect of Pb on the astrocytes was observed using immunolabeling. Increased GFAP-positive astrocytes were found in the Pb treated maternal brains; this increase or gliosis is explained as hyper-reactivity of astrocytes that attempt to provide accommodation against the toxic effect of Pb, thus protecting neurons from its hazardous effects⁶¹. The mechanism that explain the increased of GFAP-positive astrocytes and GFAP content in the neonatal brain after maternal Pb intake detected in the current study might be attributed to a potential role of cytokines, which play an important part in modulating astrogliosis. Moreover, it was described that the observed gliosis in the Pb-treated group might be caused by the formation of ROS and reduced antioxidants⁴². Similarly, studies have observed an elevated GFAP in numerous regions of the brain after Pb exposure, mainly in the hippocampus and the cerebellar cortex. Consequently, the detection of GFAP expression might probably be an

appropriate marker to determine neurodegenerative impairments⁶⁴. Regarding the fetal brains at GD20, few GFAP-positive astrocytes were occasionally seen. GFAP-positive astrocytes were detected in the cortex of the brain in the examined groups. Focally accumulated GFAP-positive astrocytes in the cortex were apparent in brains from the Pb-treated group.

Co-administration of Cur together with Pb to the mother rats produced a significant improvement, as evidenced by the study parameters and the histopathological findings in maternal and fetal brains, which appeared like the control rats, especially in the group that received the smaller dose of Pb. Similarly, it has been reported that Cur has great potential for the prevention of multiple neurological conditions⁶⁵. Also, several studies have shown that Cur exhibits protective effects against oxidative damage by exerting a potent scavenging effect for ROS and increased intracellular glutathione concentration⁶⁶. Moreover, some animal studies have shown that administration of Cur after brain ischemia has protective effects and significantly diminishes lipid peroxidation, mitochondrial dysfunction, and glial activation, as well as infarct volume; moreover, it improves cognitive deficits, neurological scores, and locomotor activity⁵¹.

Furthermore, the protective role of the antioxidant Cur is proved by a reduced MDA concentration in the brain due to Pb-caused oxidative stress⁶⁷. Suppression of SOD activity by Pb was shown in an in-vitro experiment that support the concept that Pb can cause reduced scavenging of ROS, which results in oxidative impairment. SOD activity is a significant constituent of the cellular antioxidant system that defends cells from the destructive effects of oxidants⁶⁸. Cur showed a neuroprotective effect in the different regions of the brain⁶⁹. Cur is thought to exert its anti-apoptotic and neuroprotective actions in multiple ways, including as a strong anti-inflammatory and antioxidant, and through neuromodulating activities⁷⁰. Also, it was shown that Cur exhibits a great capacity to defend brain lipids from peroxidation, improve SOD activity and shows the important inhibitory action against H₂O₂-caused cell injury^{68,71}.

CONCLUSION

This study is important in providing evidence of the beneficial role of Cur as a natural antioxidant in the protection against maternal and fetal Pb neurotoxicity. Finally, efforts with active steps should be taken towards the prevention of occupational exposure to this metal.

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