

Protective Effects of Mangiferin in Subchronic Developmental Lead-Exposed Rats

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Abstract Lead is a ubiquitous environmental and industrial pollutant. Exposure to excessive amounts of lead is especially harmful to the central nervous systems of infants and young children, and oxidative stress has been reported as a major mechanism of lead-induced toxicity. To evaluate the ameliorative potential of antioxidant mangiferin (MGN) on lead-induced toxicity, Morris water maze test, determination of blood and bone lead concentration, determination of antioxidant status in plasma, as well as observation of ultrastructural changes in the hippocampus were carried out. In the present study, under a transmission electron microscope, ameliorated morphological damages in the hippocampus were observed in MGN-treated groups. Blood and bone lead concentration in MGN-treated groups lowered to some extent ($p < 0.05$, $p < 0.01$). The activities of antioxidant enzymes, glutathione (GSH) content, and the GSH/oxidized glutathione ratio in MGN-treated groups were increased, respectively. Further studies are needed to establish whether the observed differences were a direct cause of mangiferin on lead-induced toxicity or not. This study might provide clues for the treatment of lead-induced toxicity.

Keywords Lead acetate · Mangiferin · Toxicity · Antioxidant · Reactive oxygen species

Abbreviations

B-Pb	Blood lead
CAT	Catalase
DMSA	2,3-Dimercaptosuccinic acid
DTNB	5,5-Dithiobis-2-nitrobenzoic acid
GR	Glutathione reductase
GSH	Glutathione
GSH-PX	Glutathione peroxidase
GSSG	Oxidized glutathione
GST	Glutathione-S-transferase
ICP-MS	Inductively coupled plasma mass spectrometry
LPO	Lipid peroxides
MDA	Malondialdehyde
MGN	Mangiferin
NBT	Nitroblue tetrazolium
OH	Hydroxyl radicals
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TEM	Transmission electron microscopy

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Introduction

Lead is a ubiquitous environmental and industrial pollutant that has been detected in almost all phases of biological systems. Lead has been one of the most important nonessential toxic heavy metals with wide applications for making pipes, paints, enamels, glazes, etc. [1]. Like other heavy metals, lead can be accumulated in the body [2].

Lead poisoning can affect virtually every organ system in the body. The toxic effects of lead extend from acute,

clinically obvious, symptomatic poisoning at high levels of exposure down to subclinical effects at lower levels [3]. Exposure to excessive amounts of lead is especially harmful to the central nervous systems of infants and young children: they are biologically and developmentally more vulnerable to its toxic effects. Lead may impede children's overall developmental progress by interfering with their performance in several crucial areas [4–7]. A growing convergence of results from both epidemiologic and experimental studies of lead toxicity demonstrated the adverse effects of lead on children's neurodevelopment, including reduced IQ scores and learning difficulties [8–10]. The European Food Safety Authority concluded in 2010, on the basis of the results of the Benchmark Dose (BMD) analysis, that an increase in B-Pb of 12 $\mu\text{g/L}$ (BMDL01) could decrease the IQ score by one point [11]. Because of lead's long residence time in the bone, it may liberate to pose a significant threat of delayed toxicity [12].

Lead toxicity has been known from ancient times [13], and many studies have explored the mechanisms and symptoms of this toxicity through the years [14]. Recent studies have reported lead's potential for inducing oxidative stress, and evidence is accumulating in support of the role for oxidative stress in the pathophysiology of lead poisoning [15]. Lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and weakening the antioxidant defense system of cells [16]. Oxidative stress has been reported as a major mechanism of lead-induced toxicity [17].

Treatment for lead toxicity involves the use of chelating agents, principally edetate calcium disodium, dimercaprol, penicillamine, and succimer (2,3-meso-dimercaptosuccinic acid, DMSA). Their practical applicability is limited due to their inherent toxicity at effective doses [18]. The search has been shifted toward natural products as nutraceuticals possess better health-promoting effects when compared to synthetic protectors. Thus, the importance of natural antioxidants has increased during recent years owing to their numerous pharmacological properties. Research findings have suggested that the administration of various antioxidants can prevent or subdue various toxic effects of lead and the generation of oxidative stress, in particular [17].

Mangiferin is a xanthone, and xanthones are some of the most potent antioxidants known; they are thought to be more potent than both vitamin C or vitamin E and are sometimes unofficially referred to as "super antioxidants" [19]. Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2- β -D-glucoside, MGN; Fig. 1.), generally called C-glucosyl xanthone, is predominantly found in the bark, fruits, roots, and leaves of *Mangifera indica* Linn and various other medicinal plants [19]. Mangiferin has a strong antioxidant activity in the biological peroxidation system; recently, the pharmacological functions of mangiferin in altering oxidative mechanisms have received much attention [20]. Mangiferin bears a catechol moiety, a

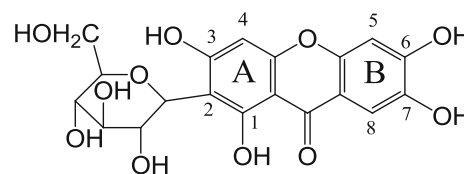


Fig. 1 Chemical structure of mangiferin (1,3,6,7-tetrahydroxyxanthone-C2- β -D-glucoside)

pharmacophore with well-established antioxidant property [21]. It is an effective antioxidant, mainly on account of its catechol moiety [22, 23], which is present also in numerous flavonoids and other polyphenols like quercetin; quercetin, meanwhile, is reported to chelate lead with its catechol moiety [16, 24].

Recent reports have shown that mangiferin protects against neuron damage caused by excitotoxic insults in vitro and prevents neuronal loss in the hippocampal CA1 area due to transient forebrain ischemia in rats and reduces neurological deficits caused by ischemic damage to the brain [25–27]. It was also shown that mangiferin has protective efficacy against heavy metal toxicity of mercury (Hg), cadmium (Cd), and arsenic (As) in in vitro and in vivo model systems [28–32].

In view of the beneficial effects of the antioxidant mangiferin on neuron damage and heavy metal-induced toxicity, we postulate that mangiferin may reduce lead-induced toxicity by lowering the lead burden and improve antioxidant status, thus ameliorating damages in the brain and improving the learning ability.

With this background, the present study has been conducted to evaluate the role of mangiferin on spatial learning and memory, lead concentration in the blood and bone, antioxidant status, as well as ultrastructure changes of the hippocampus in subchronic developmental lead-exposed rats.

Material and Method

Reagents and Material

Mangiferin was offered by the Pharmaceutical Factory, Guangxi University of Chinese Medicine, (lot. 20081217, purity >98 %). DMSA was purchased from Sigma-Aldrich, Co. (lot 051M1275V).

Nitric acid, perchloric acid, and hydrogen peroxide were of trace metal grade. Lead standard solutions 1,000 mg l^{-1} (National Standard Reference Materials, China) were used for the preparation of the working standard. Deionized Milli-Q water (Millipore, Bedford, MA, USA) was used for the preparation of all solutions. All other chemicals were of analytical grade.

Animals

We used a total of 96 weaned Wistar rats (48 males and 48 females, 26–27 days old), weighing 50–80 g, obtained from Guangxi Medical University, Nanning, China. Rats were housed in separate cages ($n=4$ per cage). Rats were kept on a 12:12-h light/dark cycle with constant ambient temperature (25 ± 1 °C) and humidity (50 ± 7 %). Food and water were available ad libitum. Experiments were also approved by the respective institutional committees for animal care and handling. All efforts were made to minimize animal suffering.

Experimental Protocol

The rats were divided into six groups ($n=16$ in each group): five groups exposed to 500 ppm of lead acetate in drinking water for 8 weeks and one group as the control group. Mangiferin (50, 100, and 200 mg/kg body weight) and DMSA were dissolved in distilled water and were orally administered to intoxicated groups for four supplemental weeks; the other intoxicated group was left as the lead-exposed group. The dosages of mangiferin were decided on the basis of experiments conducted in our own laboratory and on other published reports. The food intake and body weight of rats in all groups were weighed every 3 days and the water intake recorded every day.

Group A received distilled water (control group). Group B received lead acetate daily for 12 weeks (lead-exposed group). Group C was given DMSA (50 mg/kg body weight) by oral gavage four continual days a week for 4 weeks, starting 8 weeks after the start of lead acetate treatment until the end of the experiment. Groups D, E, and F were given mangiferin (at 50, 100, or 200 mg/kg body weight) by oral gavages daily for 4 weeks, starting 8 weeks after the start of lead acetate treatment until the end of the experiment.

After the Morris water maze test, three male rats of each group were randomly selected for transcatheter perfusion; the hippocampi were separated for sampling using a transmission electron microscope (TEM). Blood (7 mL) of the remaining rats was collected through the abdominal aorta in heparinized tubes under hydrated chloral anesthesia. Within 12 h, 5 mL blood of each animal was centrifuged at 3,000 rpm for 15 min to separate the plasma for the assessment of ROS and biochemical enzymes. The remaining 2 mL blood was kept at 4 °C for blood lead determination using inductively coupled plasma mass spectrometry (ICP-MS). The rats were euthanized by prompt dislocation of the neck vertebra and the brains removed immediately, rinsed in ice-cold saline to remove residual blood, and preserved at -80 °C in a deep freeze refrigerator for further mechanism study. The right femurs were taken out, freed from hair and muscle, and then soaked in chloroform for

24 h to defat. After that, they were dried in the oven for 12 h. The dried femurs were weighed and made into an appropriate aliquot in a crucible before the determination of lead using ICP-MS.

Morris Water Maze Test

Spatial memory was evaluated using Morris water maze. The details of Morris water maze were as described in Morris [33]. Ten rats of each group were tested for performance in a Morris water maze. The water maze consisted of a white circular galvanized tank (diameter, 200 cm; height, 50 cm; water depth, 30 cm). It was filled with tap water adjusted to 25 °C and made opaque by the addition of powdered black non-toxic tempera paint. Four locations around the edge of the pool were defined as the start points, and these divided the pool into four equal quadrants (NE, NW, SE, and SW). A circular acrylic escape platform 11 cm in diameter was placed 1 cm below the surface of the water in the middle of one of the four quadrants of the pool. The pool was placed in a 3.0×3.0-m square room containing invariant spatial stimuli. A video camera suspended from the ceiling above the middle of the tank permitted the observer to monitor the animal's behavior on a monitor placed in one corner of the room.

Place Navigation Test

Animals were placed into the tank, facing the wall of the pool, and were allowed to circumnavigate the pool in search of the escape platform for a maximum of 120 s. On each day, the start points used for each trial varied in a pseudo-random sequence such that no two trials on the same day commenced from the same start point. The time taken by animals to climb onto the hidden platform was labeled escape latency. Latency to reach the escape platform was recorded, and the animals were permitted 15 s to rest on the platform before removal from the tank. If an animal failed to locate the platform within 120 s, it was guided to the platform by the experimenter, placed on it for 10 s, and assigned a latency score of 120 s for that trial.

Spatial Probe Test

In order to determine the capability of each animal to retrieve and retain information, the platform was removed 24 h later and the rat was released into the quadrant diagonally opposite to that which contained the platform during training. The time spent in the region that previously contained the platform was labeled retention time. In each trial, the animal was quickly towel dried before being returned to the cage.

Ultrastructure

Three of the male rats in each group were given cardiac perfusion with a solution of 4 % paraformaldehyde–1 % glutaraldehyde in 0.1 M phosphate buffer. When the heart becomes white, the brains were removed immediately from the skull; pieces of selected brain regions (hippocampal CA1 area) were separated under dissecting microscope to get pieces of about 1 mm² and immediately placed into 2.5 % glutaraldehyde, kept for more than 12 h at room temperature. Samples were post-fixed in 1 % osmium tetroxide for 1 h, dehydrated in graded increasing ethanol, and embedded in epoxy resin. Polymerization was performed at 80 °C for 24 h. Blocks were cut on a Reichert ultramicrotome into ultrathin sections (60–70 nm), which were post-stained with uranyl acetate and lead citrate and viewed under a Hitachi 7650 TEM.

Determination of Lead by ICP-MS

Instrumentation

In the present paper, we attempted to determine lead concentration in the blood and femur. The general approach was to grind a sample to a powder, digest completely in a combination of hot concentrated nitric and hydrochloric acids, and analyze using ICP-MS. Measurements were performed on a Thermo Elemental X7 ICP-MS spectrometer, applying the conditions shown in Table 1; sample decomposition was accomplished in a hot block digester (Environmental Express Hot Block). All laboratory glassware was placed in a 20 % nitric acid bath for 48 h and then rinsed with several volumes of distilled double deionized water. Rinsed glassware was placed in a covered polyethylene drying basket. Glassware was stored in sealed plastic bags prior to use.

Sample Preparation Methods

A component part (0.5 mL of blood, or 400–700 mg femur) was weighed out into a labeled 50-mL digestion vessel. In a

chemical fume hood, 8 mL of concentrated nitric acid was added to each beaker and evaporated to approximately 3 mL on a hot block digester at 105±3 °C. After cooling, 2 mL of concentrated hydrochloric acid was added and stirred. Diluted with distilled water, washing the side of the beaker, to 20 mL, the solution was warmed up to at least 60 °C and gently agitated on an orbital shaker for a minimum of 4 h. It was then transferred quantitatively into a 50-mL volumetric flask and diluted to 50 mL with distilled water. Samples were diluted so that Pb results were within the calibration range of the instrument.

Analysis and Calculations

²⁰⁹Bi was used as the internal standard. A calibration blank and 0.1, 0.5, 1.0, 10.0, 20.0, and 50.0 ng mL⁻¹ of the standard solutions were used for calibration. Calibration was performed each time the instrument was set up. Lead was quantified as a sum of the signals of ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb isotopes; the total lead concentration was calculated in the component part from that of the diluted sample, accounting for all dilution, and was reported as percent by weight of the component part itself.

Biochemical Determinations

The activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), glutathione reductase (GR), and glutathione-S-transferase (GST) were assayed using commercial assay kits (Jiancheng Bioengineering Institute, Nanjing, China) in the plasma. The contents of glutathione (GSH) and oxidized glutathione (GSSG), malondialdehyde (MDA), and hydrogen peroxide (H₂O₂) were measured using commercial assay kits too.

Total SOD activity in the plasma was measured through the inhibition of NBT reduction by O²⁻ generated by the xanthine/xanthine oxidase system. One SOD activity unit was defined as the enzyme amount causing 50 % inhibition in a 1-mL reaction solution and the result expressed as units per milliliter. CAT activity in the plasma was detected using the ammonium molybdate method by measuring the intensity of a yellow complex formed by molybdate and H₂O₂ at 405 nm, after ammonium molybdate was added to terminate the H₂O₂ degradation reaction catalyzed by CAT. An enzyme activity unit was defined as the degradation of 1 mmol H₂O₂ per second per milliliter plasma; the enzyme activity was expressed as units per milliliter. GSH-Px activity was measured by quantifying the rate of H₂O₂-induced oxidation of GSH to GSSG under the existence of GSH-Px based on the modified method of 5,5-dithiobis-2-nitrobenzoic acid (DTNB). GR activity was determined by following the rate of NADPH oxidation measured as a decrease in absorbance at

Table 1 Optimum ICP-MS operating conditions

R.f power	1250 W
Coolant gas flow rate	13.0 L min ⁻¹
Auxiliary gas flow rate	0.76 L min ⁻¹
Nebulizer gas flow rate	0.84 L min ⁻¹
Sampling rate	1 mL min ⁻¹
Sampling depth	83 mm
Dwell time	50 ms
Acquisition time	7 s
Acquisition mode	Peak hopping
Collision cell technology	4.20

340 nm. One unit of GR activity was defined as 1 L plasma which consumed 1 mmol NADPH at 340 nm for 1 min. The activities of GR were expressed as units per liter. H_2O_2 in the plasma was detected by measuring the intensity of a yellow complex formed by molybdate and H_2O_2 at 405 nm.

The total GSH and GSSG levels were measured using the colorimetric microplate assay kits. The total GSH level was measured by the method of DTNB-GSSG recycling assay. The GSSG level was quantified using the same method as the total GSH assay after the plasma was pretreated with 1 % 1 mol/L 2-vinylpyridine solution to remove the reduced GSH. The amount of reduced GSH was obtained by subtracting the amount of GSSG from that of the total GSH. The content of H_2O_2 was expressed as units per liter. Plasma MDA concentration was measured using the thiobarbituric acid (TBA) method. The amount of LPO was measured as the production of MDA, which, in combination with TBA, forms a pink chromogen compound whose absorbance at 532 nm was measured. The result was expressed as nanomoles per milliliter.

All spectrophotometric measurements were carried out in a multifunctional microplate-based spectrophotometric reader (Epoch Multi-Volume Spectrophotometer System, USA).

Statistical Analysis

All statistical analysis was calculated using SPSS, version 16.0 (SPSS Inc., USA). Data were shown as the mean \pm SEM. The significance of variables among groups was tested using ANOVA, and a post hoc test was performed when needed. In all experiments, p values of <0.05 were considered to indicate statistical significance.

Results

Water Morris Maze Test

Escape latency reflects the animal spatial memory acquisition (learning ability) in place navigation test. The mean escape latency was recorded from four quadrants in 1 day. Rats' spatial learning ability was significantly increased when the training number increased and they established spatial memory via spatial learning. During the course of the experiment, the mean latency of all rats in finding the hidden platform became shorter from the first to the fourth day (Fig. 2).

The spatial probe test is a more sensitive and accurate method to test animal memory retention. For the retention times in the target quadrant, those of the rats in the lead-exposed group were shorter. For the retention time in the target quadrant, the numbers of passes over the previous target platform, the percentage of time spent in the target quadrant, those in the rats in the high-dose MGN-treated group were much more than those in others (Table 2).

Lead Concentration in Blood and Femur

The blood lead concentration in rats of the different treatment groups were presented in Table 3. The mean blood lead concentrations in lead-intoxicated rats ranged between 110.10 and 232.14 $\mu\text{g/L}$, whereas in MGN-treated rats, the ranges were between 75.09 and 230.7, 91.51 and 184.50, and 75.09 and 164.5 $\mu\text{g/L}$ in the low-, medium-, and high-dose groups, respectively. The mean lead concentration in the femur of the lead-exposed group was between 32.23 and 93.43 $\mu\text{g/g}$, whereas in the MGN-treated rats, the ranges were between 19.72 and 88.74, 25.31 and 55.48, and 16.01 and

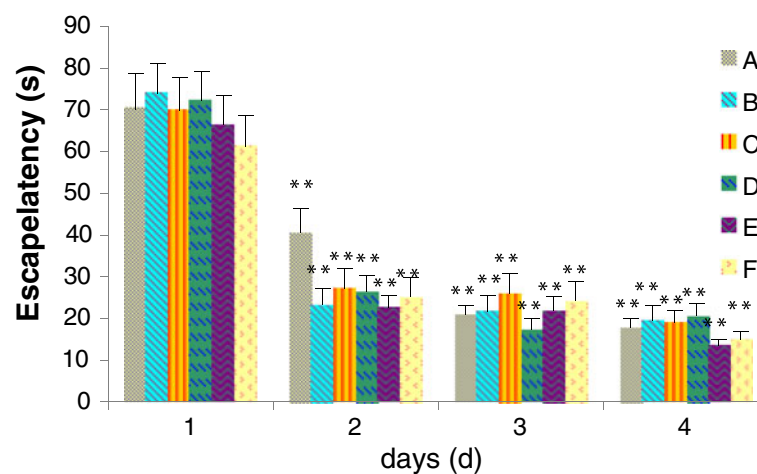


Fig. 2 Effect of mangiferin on the control and experimental animals in the place navigation test. Group A: control group; group B: lead-exposed group; group C: DMSA group; group D: low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F:

high-dose MGN-treated group. Data were presented as the mean \pm SEM, $n=10$. Statistical significance: * $p<0.05$, ** $p<0.01$ (compared with the lead-exposed group). The mean latency of all rats in finding the hidden platform became shorter from the first to the fourth day

Table 2 Effect of mangiferin on control and experimental animals in the spatial probe test

Groups	<i>n</i>	Retention time in the target quadrant(s)	No. of passes over the previous target platform (times)	Percentage of time spent in the target quadrant
A	10	42.3±1.88	4.2±0.46	0.36±0.05
B	10	39.8±2.65	3.8±0.57	0.34±0.07
C	10	40.3±2.44	3.9±0.54	0.34±0.07
D	10	42.44±2.98	3.66±0.41	0.36±0.08
E	10	42.75±4.18	3.75±0.65	0.36±0.10
F	10	44.6±3.48	4.6±0.67	0.43±0.06*

Group A: control group; group B: lead-exposed group; group C: DMSA group; group D: low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F: high-dose MGN-treated group. Data were presented as the mean±S.E.M

* $p < 0.01$ (compared with rats in the lead-exposed group)

57.80 µg/g in the low-, medium-, and high-dose groups, respectively. Concomitant use of mangiferin at the three different doses was found to reduce lead concentration (Table 3).

Morphological Changes in Hippocampal CA1 Area

Under the transmission electron microscope, in the CA1 area of the lead-exposed group, changes such as damage of the mitochondria, microfilaments, and microtubules, were observed. Vacuole formation from the swollen and distorted mitochondria, chromatin condensation, nucleolus collapse or fragmentation, and myelin sheath degeneration were found. The changes also include Golgi complex expansion, apoptotic cells, and abnormal dense bodies in the cytoplasm (Fig. 3). There was no significant change in the synaptic structure. In the low-dose MGN-treated group, there was still typical apoptosis, mitochondrial damage, and a little expansion of the Golgi, while in the medium- and high-dose MGN-treated groups, the anomaly was mild. There were still mild vascular and interstitial edemas, myelin layer, but the Golgi apparatus was no longer expanding and the mitochondria morphologically normal. No apoptotic

cells were found. The cells in the CA1 area in the hippocampus were almost normal. The study of the ultrastructure of the hippocampus revealed that mangiferin was preventive for lead-induced toxicity.

Effects of Mangiferin Treatment on Antioxidant Enzymes, Concentrations of MDA and H₂O₂, Contents of GSH and GSSG, and GSH/GSSG Ratio

SOD and CAT are two key enzymes in detoxifying intracellular O²⁻ and H₂O₂. As shown in Table 4, a significant increase of SOD activity in the plasma was observed in the high-dose MGN-treated groups, but not with the low- and medium-dose MGN-treated groups. Medium- and high-dose MGN treatment caused a significant increase in CAT activity, respectively. GSH-PX activities were significantly increased in all MGN-treated groups, while GR activity was significantly increased in the high-dose MGN-treated groups (Table 4). GST is a phase 2 enzyme that is also critically involved in the detoxification of ROS [34, 35]. Treatment with mangiferin led to a significant increase in plasma GST activity (Table 4).

As shown in Fig. 4, compared with the control group, lead exposure caused a significant increase in the concentrations of MDA and H₂O₂, while compared with the lead-exposed group, medium- and high-dose MGN treatments significantly decreased the concentrations of MDA and H₂O₂. Treatments with medium- and high-dose mangiferin resulted in a significant elevation of GSH. Changes in the GSSG content were not significant, and the GSH/GSSG ratio was increased in a dose-dependent fashion.

Table 3 Blood lead concentration and bone concentration in the control and experimental animals

Groups	<i>n</i>	Blood (µg/L)	Femur (µg/g)
A	10	29.99±4.40**	0.0088±0.0068**
B	10	159.54±35.83	58.16±15.57
C	10	97.08±44.01**	42.52±13.76**
D	10	131.80±40.74	42.94±18.92**
E	10	126.57±25.31*	41.97±9.77**
F	10	115.86±27.74**	41.35±14.83**

Group A: control group; low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F: high-dose MGN-treated group. Data were presented as the mean±SEM

* $p < 0.05$, ** $p < 0.01$ (compared with rats in the lead-exposed group)

Discussions

The term subclinical toxicity denotes the concept that a relatively low-dose exposure to lead at blood lead levels

Fig. 3 TEM images of hippocampal areas CA1 in the control and experimental animals. Group A: control group; group B: lead-exposed group; group C: DMSA group; group D: low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F: high-dose MGN-treated group. *L* lysosome, *V* vacuoles, *MYE* myelin layer, *M* mitochondria

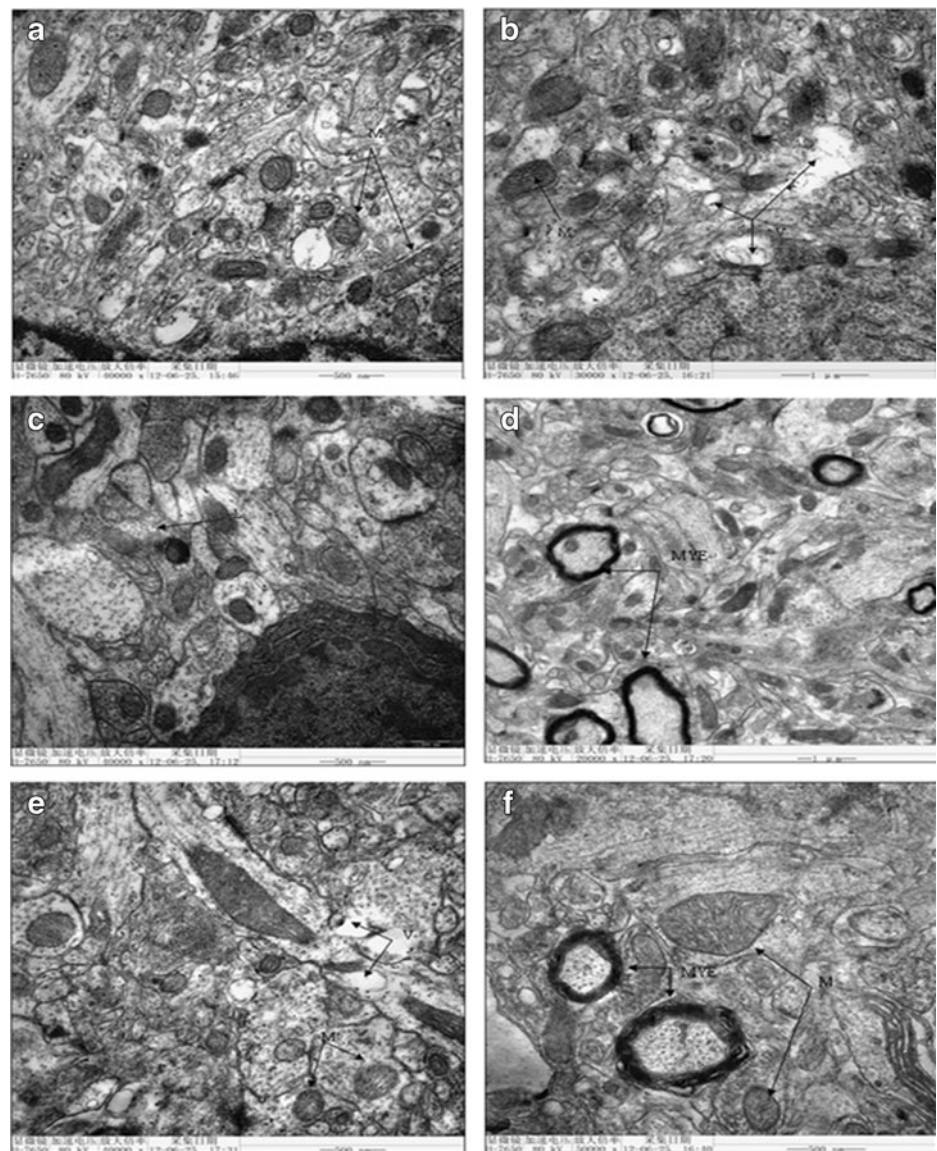


Table 4 Effect of mangiferin on SOD, CAT, GPx, GR, and GST activities in the plasma of the control and experimental animals

Groups	<i>n</i>	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)	GR (U/L)	GST (U/mL)
A	10	722.30±13.63*	16.03±2.76*	365.27±6.41	257.23±39.28	53.58±3.20*
B	10	664.88±16.23	10.35±1.54	360.83±12.22	183.28±24.54	42.53±1.20
C	10	676.51±17.73	13.16±1.49	424.16±17.93**	237.94±12.75	65.82±3.85**
D	10	684.63±21.94	13.07±0.90	470.56±18.79**	219.94±26.60	64.39±2.36**
E	10	692.01±26.00	15.77±1.95*	475.83±25.49**	237.44±18.05	75.44±4.99**
F	10	724.51±13.72*	15.26±0.88*	506.94±27.11**	261.09±22.11*	82.81±8.82**

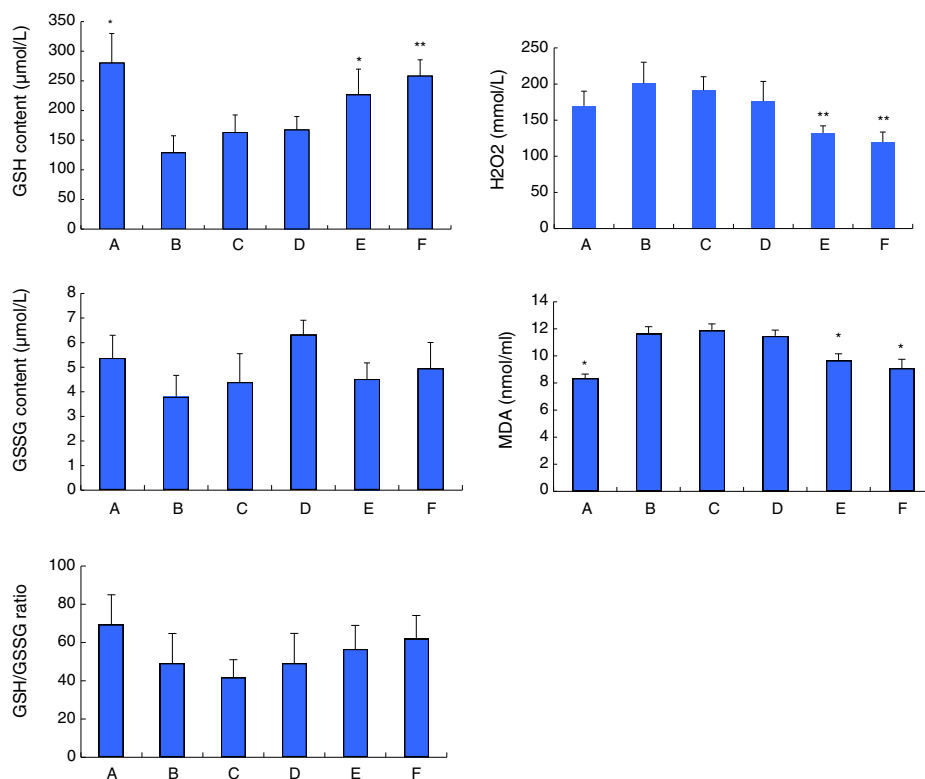
Group A: control group; group B: lead-exposed group; group C: DMSA group; group D: low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F: high-dose MGN-treated group. Data were presented as the mean±SEM. *n*=10

Units: SOD—50 % inhibition of nitroblue tetrazolium per milliliter reaction solution; CAT—micromoles of H₂O₂ degraded per second per milliliter plasma; GPx—micromoles of GSH oxidized per 5 min per 0.1 mL plasma; GR—millimoles of NADPH oxidized per minute per 1 L plasma; GST—micromoles of GSH oxidized per minute per milliliter plasma

SOD superoxide dismutase, CAT catalase, GPx glutathione peroxidase, GR glutathione reductase

p*<0.05, *p*<0.01 (compared with rats in the lead-exposed group)

Fig. 4 Effect of mangiferin on the content of glutathione (GSH), glutathione disulfide (GSSG), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and the GSH/GSSG ratio in the plasma of the control and experimental animals. Group A: control group; group B: lead-exposed group; group C: DMSA group; group D: low-dose MGN-treated group; group E: medium-dose MGN-treated group; group F: high-dose MGN-treated group. Data were presented as the mean \pm SEM, $n=10$. Statistical significance: * $p<0.05$, ** $p<0.01$ (compared with the lead-exposed group)



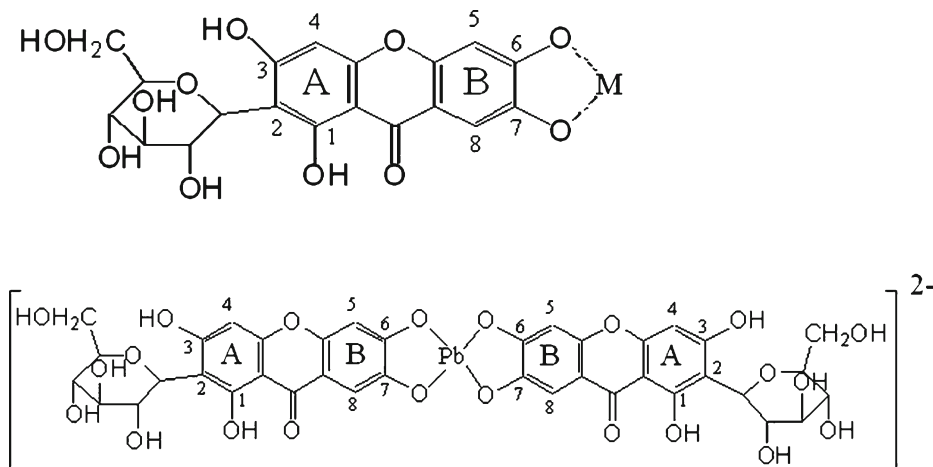
previously thought to be safe can cause harmful effects not evident in a standard clinical examination. Although they are not clinically obvious, the subclinical toxic effects of lead can be damaging [36]. In our study, the food intake and body weight between the different groups did not show significant differences; the lead-exposed rats were asymptomatic, so our animal model could be classified into subclinical lead poisoning.

Developmental exposure to lead was reported to induce learning deficits in children [37, 38] and in animal models of cognition [39–41]. Results of experiments designed to assess the performance of Pb-exposed animals on hippocampal-

dependent tasks, however, have been equivocal. The hippocampus is a central neuronal structure for learning and memory, and it plays an important role in the storage of information. In the present study, the ultrastructure of the hippocampal CA1 area showed obvious morphological damages in the lead-exposed group. Though neuropathological damage was confirmed histologically, the Morris water maze test results showed that the administration of lead acetate via the drinking water for 2 months did not affect the acquisition of spatial learning.

The term neural compensation may explain this phenomenon. Neural compensation refers to the use of alternate

Fig. 5 Structure of mangiferin showing metal chelation at the hydroxyl groups of B-ring (M=Cu²⁺, Fe³⁺, and Pb²⁺)



brain regions not normally seen in healthy young adults in order to compensate for deficits in primary avenues for successful task performance [42]. Neural compensation is not seen in healthy young adults, only in those with brain pathology [43]. The lead-exposed rats may have had incipient lead neuropathy, but somehow avoided the loss of large numbers of neurons or, alternatively, started with larger brains and more neurons and, thus, might be said to have had a greater “cognitive reserve” [44]. Therefore, they had no or very little manifestations of the disease [45], but once they become clinically impaired, probably indicating a failure of all compensatory systems.

In the Morris water maze test, rats in the high-dose group showed better performance in the spatial probe test (Table 2), which maybe due to the lowering of lead, improvement of the antioxidant status, or the increased amount of nerve growth factor secreted by human glioma cells, an action that might be involved in the observed enhancing effect of mangiferin on recognition memory [46].

In our study, compared with the lead-exposed group, the blood and bone lead concentrations in the MGN-treated group lowered. As the mangiferin dose increased, the reducing effect was more obvious. The molecular mechanisms underlying the aforementioned mangiferin effects are unknown, but recent studies with the flavonoid quercetin, a catechol containing polyphenols structurally similar to mangiferin, showed that quercetin chelates lead by forming a coordination bond with the lead through its ortho-phenolic groups located on the quercetin B-ring [16, 24]. As some studies once showed that mangiferin can form MGN-Cu²⁺ and MGN-Fe²⁺ complexes with the functional groups, namely, the hydroxyl groups of the B-ring [47–49], we speculate that mangiferin may chelate metal ion in the form shown in Fig. 5.

In this work, the increase of MDA and H₂O₂ and the decrease of antioxidant enzymes such as SOD, CAT, GSH-Px, GR, GST, the GSH level, and GSH/GSSG ratio in the plasma of the lead-exposed group suggested that the system was beginning to lose its ability to maintain the critical balance of the redox states after the 2-month exposure, although spatial learning ability impairment was not shown. Supplementation of mangiferin after lead exposure resulted in a significant induction of a scope of antioxidant enzymes, including SOD, CAT, GSH-Px, and GST, in a dose-dependent fashion. SOD, CAT, GSH, and GR replenishment on mangiferin supplementation for the entire period reflected a favorable balance between potentially harmful oxidants and protective antioxidants.

There is ample evidence in support of a very potent antioxidant activity of mangiferin [20]. Although a number of targets for antioxidant activity have been proposed, the primary antioxidant mechanism of mangiferin seems to be mediated through enhancing glutathione levels [50], scavenging of reactive oxygen species or free radicals [31],

inhibition of lipid peroxidation [30], as well as modulation of mitochondrial membrane potential [26]. As to the structure–activity relationships for the antioxidant activity of mangiferin, it has been reported that the compounds having two hydroxyl groups at the 6 and 7 positions of B generally have strong potency [51].

In summary, we have demonstrated that chronic lead exposure causes damage in the hippocampus, although no impairment in spatial learning was detected. Our findings have revealed that mangiferin ameliorated morphological damage in the hippocampus, improved the spatial learning abilities to some extent, and lowered the lead burden in blood and bone. The protective potential of mangiferin may be attributed to the quenching of the ROS generated due to oxidative stress induced by lead, normalization of antioxidant levels, and chelation to metal ions. Further studies are needed to establish whether the observed differences were a direct cause of mangiferin on lead-induced toxicity or not. This study might provide clues for the treatment of lead-induced toxicity.

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