BENEFICIAL ROLE OF QUERCETIN ON DEVELOPMENTAL BRAIN OF RATS AGAINST OXIDATIVE STRESS-INDUCED BY LEAD POISONING

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Summary

Considering the vulnerability of the developing brain to lead (Pb) neurotoxicity, this study was carried out to investigate the plausible protective effect of quercetin on Pb-induced poisoning of rat's developing brain tissues and antioxidant enzymes activities. Wistar dams were exposed to 600 ppm of Pb-acetate contaminated drinking water during pregnancy and lactation. Positive control group of rats received quercetin (QCT, 25 mg/kg/day, i.p.) daily for 3 days prior to lead exposure, and QCT administration continued for further consecutive 15 days. Biochemical variables indicative of oxidative stress along with lead poisoning were determined in blood, cerebellum and hippocampus of dams and pups after euthanization. Exposure to lead caused a significant increase ($p<0.05$) in plasma and brain Pb concentrations of both dams and weaned pups. Moreover, there was a pronounced depletion in the activities of glutathione (GSH), δ-aminolevulinic acid dehydratase (ALAD), glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) in the cerebellum and hippocampus, and significant increase ($p<0.05$) in malondialdehyde (MDA). In QCT-treated rats, CAT, GSH, SOD, GSH-Px, and ALA-D significantly increased ($p<0.05$). Data obtained suggests that Pb exposure led to varying degrees of oxidative stress in rat's developing brain regions, and that QCT treatment ameliorated the oxidative stress.

Keywords: Lead Poisoning, Oxidative Stress, Quercetin, Cerebellum and Hippocampus, Antioxidants.

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Introduction

Quercetin, a member of the flavonoids family, occurs commonly, and is widespread, in the plant kingdom. Flavonoids are plant polyphenolics found as pigments in leaves, barks, seeds, fruits and flowers, and they are often closely associated with vitamin C, to which they offer synergistic effects[1]. The pharmacological effects of many herbal drugs (phytomedicines) may be ascribed to their flavonoid constituents, which inhibit certain enzymes and possess antioxidant activities. Quercetin (3,3´,4´,5-7-pentahydroxyflavone), is a flavonol chemically related to kaemferol.

Quercetin prevents oxidantive injury and cell death by several mechanisms, including scavenging oxygen radicals, inhibiting xanthine oxidase, lipid peroxidation, and chelating metal ions[2].

Lead (Pb) poisoning is still considered a primary environmental hazard to children. Pb exposure during pregnancy and in young children is of particular concern because the developing nervous system is especially susceptible to Pb toxicity. Developing fetuses and young children absorb Pb more readily than adults, and Pb enters the central nervous system (CNS) quite freely, although the exact mechanisms have not been elucidated[3]. Exposure to Pb during neurodevelopment has significant effects on neurobehavioral and intellectual performance, also resulting in attention, hyperactivity, and learning disorders[4]. There is no proven safe lower limit for Pb exposure; toxic effects have been observed in children with blood lead levels (BLLs) of less than 0.48 μM. Subtle neurobehavioral dysfunction and decline in intelligence quotient (IQ) in children have been associated with an overall median BLL of 0.24 μM[5]. Lead has been shown to be neurotoxic during neural differentiation and synaptogenesis. However, Pb seems to produce its greatest toxic effect during the later stages of brain development, perhaps by interfering with the trimming or pruning of synaptic connections and apoptosis[6]. Lead can also produce significant decreases in the formation of myelin, particularly during late gestational development and during the postnatal period. Within the developing brain, Pb-induced damage occurs preferentially in the prefrontal cerebral cortex, hippocampus, and cerebellum. Lead toxicity may damage the basal forebrain and the primary visual cortex, and cause changes in the permeability of capillaries in the cerebral cortex[7]. The hippocampus, a critical neural structure for learning and memory, is also affected by low-level inorganic Pb and organolead exposure. The cerebellum primarily controls motor movements, and may be partially responsible for speech, learning, emotions, and attention. A study on six-year-old children exposed to Pb at 1-5 years of age indicated postural disequilibria[8].

Cells in the mammalian body have developed various antioxidant defense systems against free radical attacks. Glutathione (GSH), and γ-glutamyl-cysteinyl-glycine, play a major role in protecting body cells against oxidative stress[9]. Glutathione reductase (GR) reduces glutathione disulfide (GSSG) to GSH, thereby supporting the antioxidant defense system. Other antioxidant enzymes, which remove peroxides, and superoxide radicals from the body, including glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD), are also potential target of lead[9]. Because GSH-Px requires selenium for its activity, when lead forms a complex with selenium, GSH-Px activity decreases. Lead is known to inhibit haeme synthesis, and since CAT is a haeme-containing enzyme, it causes CAT activity to decrease. SOD dismutases O_2^- and requires copper and zinc for its activity. Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation, whereas zinc ions seem to stabilize the enzyme. However, recent studies suggest oxidative stress as one of the important mechanisms of toxic effects of lead[10]. In view of this, the aim of the present study was: to examine the accompanying morphological changes and antioxidant status in the brain areas mostly affected by the lead toxicity.

Materials and Methods

Animal care and monitoring

Forty healthy, female, Wistar rats weighing 250–300 g (averaging 12 weeks old) were used in this study. They were housed under standard laboratory conditions of light, temperature (21±2^{o}C) and relative humidity (55±5%). The animals were given standard rat pellets and tap water *ad libitum*. The rats were randomly divided into 2 experimental groups of 20 rats each: Group A (positive control that receive quercetin and later exposed to Pb), and Group B (Pb-exposed group). Maintenance and treatment of the animals were in accordance with the principles of the *"Guide for care and use of laboratory animals in research and teaching"* prepared by the National Institutes of Health (NIH) publication 86-23, revised in 1985.

Impregnation and gestation monitoring procedures

Group A animals received quercetin (QCT, 25 mg/kg/day, i.p.) daily for 3 days prior to Pb-exposure, and QCT treatment continued for further 15 days. Each animal was thereafter injected with 50 μg of luteinizing hormone-releasing hormone (LHRH). The LHRH was contained in a 0.1% bovine serum albumin solution with the total injected volume of the active hormone being 0.2 ml per animal[11]. The LHRH primes for estrus to occur during the 12-h dark cycle, and the animals were allowed to mate with male rats during the 12-h dark cycle. After the mating period, each female was evaluated for vaginal sperm plug and a positive vaginal smear. Positive evaluations were regarded as day 0 of gestation (GD0). No animal was allowed to mate more than once during the course of the study; and any animal that did not become pregnant was removed from the study.

Animal exposure and offspring outcome

Pregnant rats (groups A and B) were exposed to 600 ppm of Pb-acetate in drinking water during pregnancy and lactation, but were still kept in separate cages. The mothers (dams) were then allowed to birth their pups naturally, and 8-h postpartum, the neonates were weighed and evaluated for any gross morphological abnormalities. All animals were allowed to nurse freely and were weaned at 21 postnatal days of age (PND21). Maternal body weights were measured on a weekly basis throughout pregnancy and lactation. On PND21, neonates were separated from dams and housed with littermates, but pups from group A were still separated from group B pups (five per cage). In addition, pups' weights at birth and during the first 3 weeks of age were also recorded on a weekly basis.

Tissue collection for lead analyses

In order to control the level of exposure of each litter, dams were euthanized a week after weaning. Dams of different groups were sacrificed under pentobarbital sodium (60 mg/kg, intraperitoneally) anaesthesia and decapitated. Their brains were dissected immediately. The two brain regions of interest; viz cerebellum and hippocampus, were excised, processed immediately for biochemical investigation and the remaining brain tissue was stored at -20^{o}C for wet digestion and estimation of Pb^{2+} content. The pups were also sacrificed at different time intervals until the end of study period. Due to the quantity of the tissue, tissues of four littermates were pooled. The abdomen of each rat was opened and blood samples collected by cardiac puncture for Pb and δ-aminolevulenic acid dehydratase (ALA-D) determinations.

Milk Collection

Milk was collected from both Pb-treated and Pb + QCT-treated dams on PND5, PND10, and PND15 by a modification of the method of Osakarsson *et al*;[12]. Briefly, the pups were separated from dams for approximately 4-h before milk collection; to allow milk to accumulate. Dams were sedated with ketamine (100 mg/kg i.p.) and were given oxytocin (1 IU i.p.; 0.2ml of a 5 IU solution) about 5 min before collection. Milk was expressed manually into clean vials, and the samples were weighed and stored at -20^{o}C until processed for Pb analysis.

Preparation of brain homogenates

The rats were sacrificed by decapitation under light anesthesia. Cerebellum and hippocampus were quickly dissected, weighed and placed in ice-cold physiological saline, and were later homogenized with Potter Elvehjem homogenizer. 10% homogenates were prepared in 6.7 mM phosphate buffer, pH 7.4, and centrifuged at 10,000 rpm for 10 min at 4^{o}C, and the supernatant was used for antioxidant enzyme assays. For the determination of lipid peroxidation, brain tissues were homogenized in 1.15% KCl solution to obtain a 10% (w/v) homogenate. Protein content of the brain homogenates was determined by using bicinchoninic acid (BCA) protein assay reagent (Pierce Chemical Company, Rockford, IL, USA)[13].

Blood δ-aminolevulinic acid dehydratase activity (ALA-D)

ALA-D activity was assayed by measuring the rate of product (porphobilinogen) formation, according to the method of Sassa[14]. 0.2 ml of heparinized blood was mixed with 1.3 ml of distilled water and incubated for 10 min at 37^{o}C for complete haemolysis. After adding 1 ml of standard δ-ALA (δ-aminolevulinic acid), the tubes were incubated for 60 min at 37^{o}C. Enzyme activity was stopped after 1-h by adding 1 ml of 10% trichloroacetic acid (TCA). After centrifugation (1500 x g) of the reaction mixture, an equal volume of Ehrlich reagent was added to the supernatant, and the absorbance was recorded at 555 nm after 5 min. ALA-D activity is reported as nmol porphobilinogen formed h^{-1} ml erythrocytes^{-1} for blood enzyme.

Tissue δ-aminolevulinic acid dehydratase activity (ALA-D)

Brain ALA-D activity was determined following the method of Goering *et al*;[15]. A 20% tissue homogenate (w/v) was prepared in 0.05 M Tris-Cl (pH7.4) and 0.25 M sucrose. For estimation of ALA-D, 0.2 ml of tissue homogenate was mixed with 1.3 ml of distilled water. After adding 1 ml of standard δ-ALA (δ-aminolevulinic acid), the tubes were incubated for 60 min at 37^{o}C. Enzyme activity was stopped at 1-h by adding 1 ml of 10% trichloroacetic acid (TCA). After centrifugation (1500 x g) of the reaction mixture, an equal volume of Ehrlich reagent was added to the supernatant, and the absorbance was recorded at 555 nm after 5 min. ALA-D activity is reported as nmol porphobilinogen formed h^{-1} mg protein^{-1} for cerebellar and hippocampus enzyme.

Catalase activity (CAT)

The activity of catalase (CAT) was measured using its perioxidatic function according to the method of Johansson and Borg[16]. 50 µL potassium phosphate buffer (250 mM, pH 7.0) was incubated with 50 µl methanol and 10 µL hydrogen peroxide (0.27%).

The reaction was initiated by addition of 100 µL of enzyme sample with continuous shaking at room temperature (20°C). After 20 minutes, the reaction was terminated by addition of 50 µL of 7.8 M potassium hydroxide. 100 µL of purpald (4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole, 34.2 mM in 480 mM HCl) was immediately added, and the mixture was again incubated for 10 minutes at 20°C with continuous shaking. Potassium peroxidate (50 µL 65.2 mM) was added to obtain a coloured compound. The absorbance was read at 550 nm in a spectrophotometer. Results are expressed as micromoles of formaldehyde produced/mg protein.

Reduced GSH and oxidized glutathione GSSG activities

Brain GSH and GSSG contents were measured as described by Hissin and Hilf [17]. For measuring GSH contents, 4 ml of pancreatic homogenate was precipitated by adding 1 ml of 25% metaphosphoric acid and centrifuged at 10,000 x g (Ultracentrifuge, Hitachi, Japan) for 30 min. Supernatant was diluted 20 times with same buffer, and 100 µl of OPT (O-pthaldehyde) was added. In addition, for GSSG assay, 0.5 ml supernatant was incubated at room temperature with 200 µL of 0.04 mol/l N-ethylmaleimide solution for 30 min and to this mixture 4.3 ml of 0.1 mol/l NaOH was added. A 100 µl sample of this mixture was taken for the measurement of GSSH using the procedure described above for GSH assay, except that 0.1 mol/L NaOH was used as the diluent instead of phosphate buffer. Samples were incubated at room temperature for 15 min and fluorescence was measured at 350 nm (E_x)/420 nm (E_m) with Tecan Spectra Fluor Plus (Germany).

Superoxide dismutase activity (SOD)

Brain SOD activity was assayed by the method of Kakkar *et al*;[18]. Reaction mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.0), 0.1 ml of phenazine methosulphate (PMS) (186 µM), 0.3 ml of nitro blue tetrazolium (NBT) (300 µM). 0.2 ml of the supernatant obtained after centrifugation (1500 x g, 10 min followed by 10,000 x g, 15 min) of 5% brain homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 µM) and stopped precisely after 1 min by adding 1 ml of glacial acetic acid. The amount of chromogen formed was measured by recording colour intensity at 560 nm. Results are expressed as units/mg protein.

Glutathione peroxidase activity (GSH-Px)

Glutathione peroxidase (GSH-Px) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide method of Flohe and Gunzler[19]. Supernatant obtained after centrifuging 5% brain homogenate at 1500 x g for 10 min, followed by 10,000 x g for 30 min at 4°C, was used for GSH-Px assay. One milliliter of the reaction mixture containing 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of H_2O_2 (1 mM) and 0.3 ml of brain supernatant was prepared. After incubation at 37°C for 15 min, the reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500 x g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of the reaction supernatant. After mixing, absorbance was recorded at 420 nm. One unit of enzyme activity was defined as the amount of enzyme that transformed 1 µmol of NADPH to NADP per minute. Results are expressed as units/mg protein.

Lipid peroxidation contents (LPO)

Brain lipid peroxidation was measured by the method of Ohkawa *et al*;[20]. The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) was determined in the reaction. For each sample assayed, four tubes were set-up containing 100, 150, 200 and 250 µL of tissue homogenate, 100 µL of 8.1% sodium dodecyl sulphate solution (SDS), 750 µL of 20% acetic acid, and 750 µL of 0.8% aqueous solution of TBA (w/v). The volume was made up to 4 ml with distilled water, mixed thoroughly and heated at 95^{o}C for 60 minutes. After cooling, 4 ml of n-butanol was added to each tube, the contents was mixed thoroughly, and then centrifuged at 3000 rpm for 10 minutes. The absorption of the clear, upper (n-butanol) layer was read at 532 nm. 1,1,3,3 tetraethoxy propane (97%) was used as the external standard. Results are expressed as nmol TBARS/mg tissue.

Elemental analysis

Lead concentrations in the blood, milk and brains of the animals were measured after wet digestion, using Microwave Digestion System (CEM, USA, model MDS-2100). Samples were brought to a constant volume and determination of blood, milk and brain tissue lead concentrations was performed using an auto sampler (AS-72) and graphite furnace (MH) fitted with an atomic absorption spectrophotometer (AAS, Perkin Elmer model AANalyst 100), method of Oteiza *et al*;[21]. The digested solutions were quantitatively transferred to volumetric flasks and diluted to 10 ml with ultrapure water (Milli-Q OM-140 deionization system). Certified reference solutions (1000 mg metal/L; Fisher Scientific, Santa Clara, CA) were used to generate standard curves for each sample. Standards for all determinations were prepared freshly daily from a Pb nitrate AA working stock solution in 0.5% Ultrex HNO_3.

Statistical Analysis

Data obtained were expressed as means (±SEM), and analyzed using repeated measures of variance. The differences between the means were analyzed statistically with one-way analysis of variance (ANOVA; 95% confidence interval). Values of $p<0.05$ were taken to imply statistical significance.

Results

Body weight of dams and pups

Body weights of the animals (239±6 g) were not altered by Pb acetate treatment as shown in Fig. 1. Fluctuations that occur in body weight during the pregnancy and lactation periods were physiological. Profile analysis also showed that the Pb regimen did not influence ($p>0.05$) the weight of pups (5.8±0.2 g) either at birth, or during the first 3 weeks after birth (Fig. 1). Pb acetate has no effects on brain weight of dams and pups (1.91±5 g), (0.256±0.5 g), respectively. The treatments also had no effect on organ-to-body weight ratios of pups' brain (27.7 mg/g).

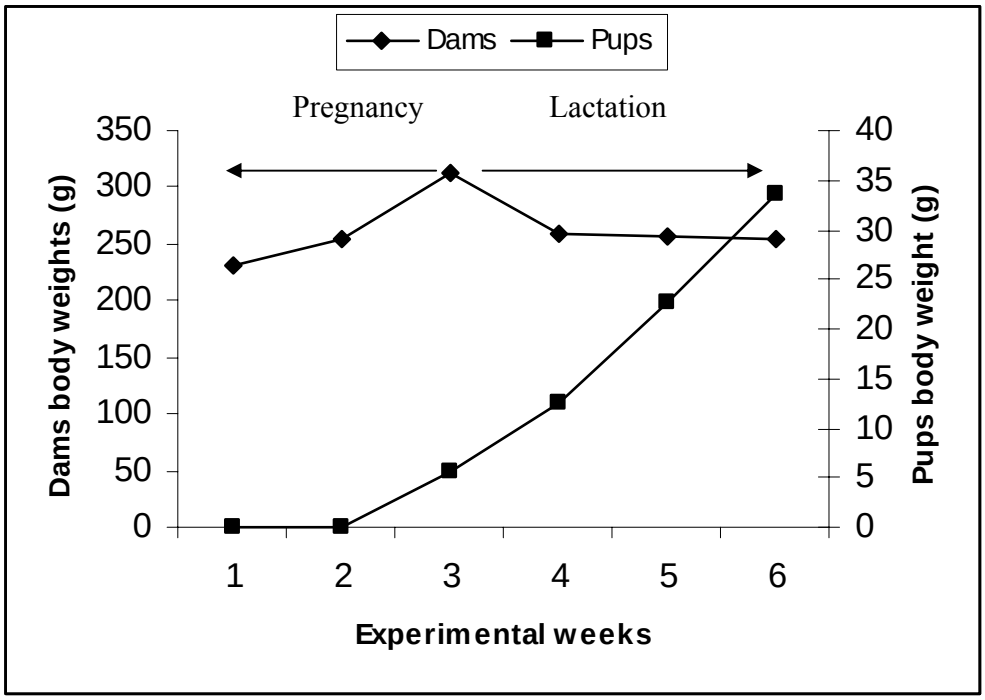

Figure 1. Changes in dams and pups body weights: The figure illustrates fluctuations in dams' weight due to conception, the pups weight gain was normal with aging

Lead concentration in blood, milk and brain tissue

Blood Pb concentration varied widely among Pb-exposed and Pb + QCT-treated groups. Blood lead levels (BLLs) significantly decreased ($p<0.05$) in QCT-treated dams (0.413±0.13 μM) when compared to Pb-exposed dams (4.488±0.17 μM) and normal rats (not detected). The concentration of Pb in the brain of Pb-exposed dams was significantly higher ($p<0.05$) than respective QCT-treated dams (Fig. 2). Elevated concentrations of lead in milk from lactating dams were related to exposure concentration and this can contribute to the neonatal lead burden before weaning. Breast milk lead concentrations decreased significantly ($p<0.05$) in QCT-treated dams (0.159±0.18 μM) as compared to Pb-exposed dams (2.694±0.16 μM). The pups of QCT-treated dams also have significant lower ($p<0.05$) BLLs (0.229±0.19 μM) as compared to pups of Pb-exposed group (2.311±0.12 μM). Brain tissue Pb concentrations in the pups from Pb-exposed dams were significantly higher ($p<0.05$) than the pups from Pb + QCT-treated dams (Fig. 2). In both dams and pups, lead concentrations in the hippocampus were persistently higher than those in the cerebellum, blood and milk. Lead-induced disruption of the prooxidant/antioxidant balance could induce injury via oxidative damage to essential biomolecules such as protein, lipids and DNA[22]. Lead irreversibly binds to the sulfhydryl (-SH) group of proteins, resulting in structural and functional alterations in the nervous system.

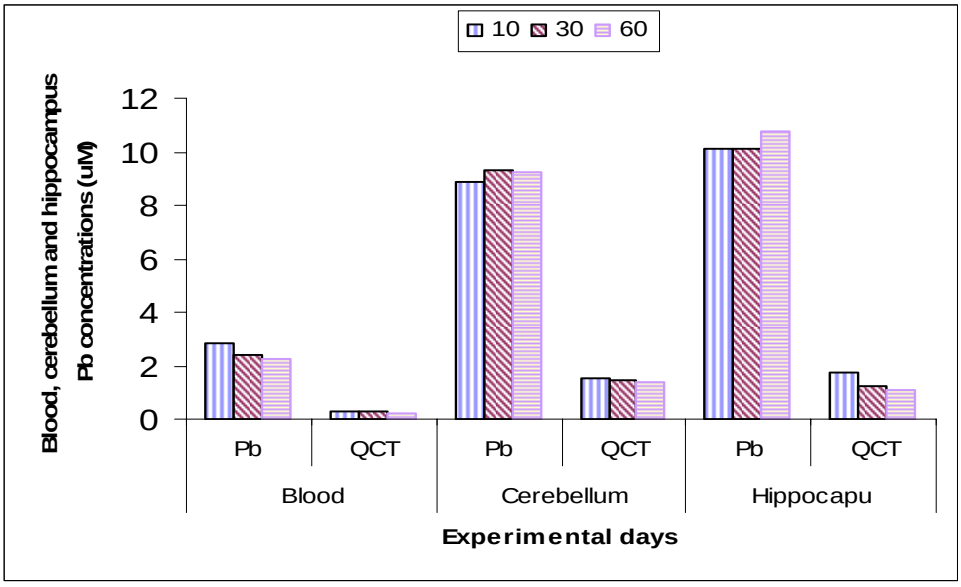

Figure 2. Lead concentration (μM) in blood and brain tissue of pups of lead-exposed and lead + QCT- treated dams. Pb concentration was higher in the hippocampus of the pups than blood and cerebellum, but with time, responded to QCT treatment better than cerebellum

Blood and tissue δ-aminolevulinic acid dehydratase Activity (ALAD)

Tables 1 and 2 shows the effects of lead on brain and blood ALAD activity in Pb-exposed in comparison with QCT-treated pups. Lead exposure caused significant depletion ($p<0.05$) of blood ALAD activity (129.24±0.19) when compared with normal rats (174.48±0.21 data not shown), whereas a significant protection of blood ALAD activity was noted in dams treated with QCT (174.56±0.39). Inhibition of nearly 70% in the red blood cell ALAD activity was observed in the pups of Pb-exposed dams. ALAD activity in brain of dams exposed to Pb showed significantly lower ($p<0.05$) enzyme level (4.49±0.53) as compared to normal rats (7.98±0.31 data not shown), whereas administration of QCT restored altered brain ALAD activity to (7.95±0.31), in the dams. ALAD activity was more inhibited in the pups than in the dams, both in blood and brain. Consequently, ALAD inhibition can impair heme biosynthesis and can results in the accumulation of ALA, which may disturb the aerobic metabolism and also have some pro-oxidant activity[14]. In the present study, accumulation of ALA during chronic Pb-exposure, resulting from inhibited ALAD activity, may undergo metal catalyzed auto-oxidation, resulting in the conversion of oxyhemoglobin to methemoglobin in a process that appears to involve the formation of reactive oxygen species such as superoxide and hydroperoxides[23].

Lipid Peroxidation contents (LPO)

Table 1 and 2 shows the MDA concentration, an end-product indicative of the extent of lipid peroxidation, in the cerebellum and hippocampus. The lipid peroxide level (152±13) in the brain of dams exposed to lead was significantly higher ($p<0.05$) than the positive control (78±14) value.

Treatment with QCT significantly lowered ($p<0.05$) the MDA level in the brain, indicating therapeutic efficacy of QCT in reversing the lipid peroxidation induced by lead. The concentration of thiobarbituric acid-reactive substances (TBARS), which is a reflection of endogenous lipid oxidation level, was also higher in the brain of pups of lead-exposed animals (Tables 1 and 2). Numerous studies in the past, have suggested that lead causes hemolysis and lipid peroxidation[24]. Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effects induced by oxidative stress, and occurs readily in the tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids[25,26].

Activity of Antioxidant enzymes

The effect of Pb exposure during pregnancy and lactation on the antioxidant enzyme activity of cerebellum and hippocampus is shown in Tables 1 and 2. Brain GSH, SOD, CAT and GSH-Px activities decreased significantly ($p<0.05$) following lead-exposure. On the order hand, the dams that received QCT treatment showed significant protection to GSH, SOD, CAT and GSH-Px levels. Antioxidant enzyme activity in the littermates of Pb-exposed dams was significantly lowered when compared with group that received QCT treatment (Tables 1 and 2). The mechanism resulting in the formation of free radicals includes generation of superoxide ion[27]. In our present study, we observed a significant decrease in the activity of brain GSH and SOD free radical scavengers. This could be due to an increased lead concentration in these tissues and its possible interaction with this enzyme, thereby reducing the disposal of superoxide radicals. Lead-induced decrease in brain GSH-Px activity may arise as a consequence of impaired functional groups such as GSH and NADPH, or selenium mediated detoxification of toxic metals[28,29]. Lead also decreased CAT activity, since CAT is a heme-containing enzyme, and lead inhibit heme synthesis.

Table 1. Antioxidant enzymes activity in the cerebellum of 23 and 60-day-old pups of Pb-exposed and Pb + QCT-treated dams

	23-day-old pups		60-day-old pups	
Parameters	Exposed	QCT-treated	Exposed	QCT-treated
CAT (μmol/mg protein)	0.24±0.4[a]	0.52±0.3	0.21±0.5[a]	0.58±0.2
GSH (U/mg protein)	2.76±1.1[a]	6.53±0.6	3.12±0.2[a]	5.06±0.3
SOD (U/mg protein)	17.4±1.5[a]	29.8±1.2	13.4±1.7[a]	32.2±1.3
GSH-Px (U/mg protein)	0.32±1.2[a]	0.57±1.7	0.35±1.3[a]	0.59±1.5
MDA (nmol/mg protein)	136±13[b]	86±18	137±10[b]	87±15
ALA-D (Brain nmol/mg protein)	4.71±0.21[a]	7.97±0.18	4.51±0.23[a]	8.14±0.19
ALA-D (Blood nmol/mg Protein)	117.12±0.3[a]	159.14±0.6	119.13±0.7[a]	161.13±0. 7

Data are presented as means (±SEM) of 10 rats for all groups. [a] Significant decrease ($p<0.05$) and [b]Significantly higher ($p<0.05$) in Pb-exposed when compared with lead + QCT-treated groups.

Table 2. Antioxidant enzymes activity in the hippocampus of 23 and 60-day-old pups of Pb-exposed and Pb + QCT-treated dams

	23-day-old pups		60-day-old pups	
Parameters	Exposed	QCT-treated	Exposed	QCT-treated
CAT (μmol/mg protein)	0.22±0.1[a]	0.59±0.3	0.26±0.4[a]	0.61±0.4
GSH (U/mg protein)	3.19±0.5[a]	5.62±0.6	3.09±0.7[a]	5.47±0.6
SOD (U/mg protein)	19.2±0.7[a]	32.3±1.5	17.3±1.4[a]	31.2±1.1
GSH-Px (U/mg protein)	0.30±0.1[a]	0.59±1.3	0.32±1.1[a]	0.62±1.2
MDA (nmol/mg protein)	139±11[b]	79±13	141±14[b]	81±16
ALA-D (Brain nmol/mg protein)	3.92±0.6[a]	7.98±0.5	3.88±0.7[a]	8.32±0.3
ALA-D (Blood nmol/mg Protein)	119.18±0.1[a]	166.21±0.4	122.17±0.2[a]	168.12±0.1

Data are presented as means (±SEM) of 10 rats for all groups. [a] Significant decrease ($p<0.05$) and [b]Significantly higher ($p<0.05$) in Pb-exposed when compared with lead + QCT-treated groups.

Discussion

The aim of the present study was to evaluate the amelioration of querctin on oxidative stress induced by lead poisoning at developmental stage of cerebellum and hippocampus. Lead is an element in the form of a divalent cation, and among all the organs which lead has its toxic effects; the brain is the most sensitive to these effects. Although all of lead's toxic effects cannot be tied together by a single unifying mechanism, lead's ability to substitute for calcium and perhaps zinc is common to many of its toxic actions[30]. Lead's ability to pass through blood brain barrier (BBB) is due in large part to its ability to substitute for calcium ions (Ca^{2+}). Experiments with metabolic inhibitors suggest that back-transport of lead via the Ca-ATPase pump plays an important role in this process[31]. Certainly, the effects of lead on second messengers, transmitter release, transport of thyroid hormone would alter normal neuronal development as seen in, for example, volumetric changes in the developing hippocampus[32], morphological changes in the developing cortex[33] of lead-exposed rats and altered dendritic branching of cerebellar Purkinje cells in postnatally exposed kittens[34].

Lead induced disruption of the prooxidant/antioxidant balance could induce injury via oxidative damage to critical biomolecules[22]. Lead irreversibly binds to the sulfhydryl (-SH) group of proteins, causing impaired functions. δ-aminolevulinic acid dehydratase (ALA-D) is sulfhydryl-containing enzyme that catalyzes the asymmetric condensation of two δ-aminolevulinic acid (ALA) molecules yielding porphobilinogen, a heme precursor[35]. Consequently, ALA-D inhibition can impair heme biosynthesis[14] and can results in the accumulation of ALA, which may disturb the aerobic metabolism and also have some pro-oxidant activity[36].

In the present study, accumulation of ALA during chronic Pb-exposure, resulting from inhibited ALAD activity, may undergo metal catalyzed auto-oxidation, resulting in the conversion of oxyhemoglobin to methemoglobin in a process that appears to involve the formation of reactive oxygen species such as superoxide and hydroperoxides[23]. The conversion of ALA keto form into ALA enol form is necessary for auto-oxidation reaction because levulenic acid, without the amino group that is thought to facilitate the enolization, has not been found to be active in oxidation reaction[37,38,39].

The mechanism resulting in the formation of free radicals includes generation of superoxide ion. In our present study, we observed a significant decrease in the activity of brain SOD[27], a free radical scavenger and metalloenzyme (zinc/copper). This could be due to an increased lead concentration in these tissues and its possible interaction with this enzyme[40], thereby reducing the disposal of superoxide radicals. Lead-induced decrease in brain GSH-Px activity may arise as a consequence of impaired functional groups such as GSH and NADPH, or selenium mediated detoxification of toxic metals[28]. Lead also decreased CAT activity, since CAT is a heme-containing enzyme, and lead inhibit heme synthesis. The concentration of thiobarbituric acid-reactive substances (TBARS), which is a reflection of endogenous lipid oxidation level, was also higher in the brain of lead-exposed animals. Numerous studies in the past, have suggested that lead causes hemolysis and lipid peroxidation[24]. Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effects induced by oxidative stress, and occurs readily in the tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids[25]. The interaction of lead with oxyhemoglobin (oxyHb) has been suggested to be an important source of superoxide radical formation in RBCs. This might perhaps be the reason for lead induced substantial increase in the autooxidation of hemoglobin in an *in vitro* liposome model. Ercal *et al*;[41] recently postulated that antioxidant enzymes inhibited hemoglobin autooxidation by lead, suggesting O_2^- and H_2O_2 were involved in this process. It can thus be concluded that lead induced oxidative stress might be due to the interaction of lead with oxyhemoglobin, leading to peroxidative hemolysis in RBC membranes. RBCs have a high affinity for lead and they contain a majority of lead found in the blood stream, hence, they are vulnerable to oxidative damage and many other cells. Lead causes damages by impairing the antioxidant defense system.

Quercetin, a key representative molecule of the flavonoid group, has been suggested to have beneficial effects such as antitumoral, antithrombotic, anti-inflammatory and antiapoptotic effects, as well as effects inhibiting platelet aggregation and the growth of certain types of cancer[42]. In our present study, quercetin provided recovery in the altered biochemical variables, and restored depleted ALA-D activity in blood and brain to normal. This may be attributed to its active participation in intracellular signaling, and inhibition of several steps involved in the activation of intracellular metabolic events that leads to the generation of oxygen free radicals. Self-maintained oxidative reactions have been identified in arteriosclerosis, the main pathological condition leading to cerebrovascular, as well as neurodegenerative disease[43]. Quercetin proved to be significantly better in restoring the altered activity of antioxidant enzymes like GSH, SOD, CAT, GSH-Px and MDA towards their normal values in blood and brain. This role of QCT, especially in decreasing oxidative stress in brain and blood by directly scavenging ROS, might be exerted via its sulfhydryl group.

These results are supported by the data on lead concentrations seen in rats that received quercetin. This could be explained by the fact that QCT block the initial mitochondrial metabolic failure usually initiated by lead, which will eventually lead to disruption of ionic pump functioning at the membrane level. This step also blocks massive neurotransmitter and glutamate release, which usually increase calcium in lead-exposed cells.

Based on our findings, we conclude that lead poisoning is associated with oxidative stress in blood and brain tissues. Nevertheless, quercetin exhibited antioxidant activity, and is able to diminish and/or prevent biochemical derangements produced by Pb poisoning.

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