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Article *in* Journal of Physiology and Biochemistry · June 2010

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J Physiol Biochem (2010) 66:143–151
DOI 10.1007/s13105-010-0019-5

ORIGINAL PAPER

Protective effect of green tea on lead-induced oxidative damage in rat's blood and brain tissue homogenates

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Received: 7 March 2010 / Accepted: 30 April 2010 / Published online: 1 June 2010

Abstract Recent studies have shown that lead (Pb) could disrupt tissue prooxidant/antioxidant balance which lead to physiological dysfunction. Natural antioxidants are particularly useful in such situation. Current study was designed to investigate efficacy of green tea extract (GTE), on oxidative status in brain tissue and blood caused by chronic oral Pb administration in rats. Four groups of adult male rats (each 15 rats) were utilized: control group; GTE-group (oral 1.5% *w*/*v* GTE for 6 weeks); Pb-group (oral 0.4% lead acetate for 6 weeks), and Pb+GTE-group (1.5% GTE and 0.4% lead acetate for 6 weeks). Levels of prooxidant/antioxidant parameters [lipid peroxides (LPO), nitric oxides (NO), total antioxidant capacity (TAC), glutathione (GSH), glutathione-*S*-transferase (GST), superoxide dismutase (SOD)] in plasma, erythrocytes, and brain tissue homogenate were measured using colorimetric methods. Pb concentrations in whole blood and brain tissue homogenate were measured by atomic absorption. In Pb-group, levels of LPO were higher while NO and GSH were lower in plasma, erythrocytes, and brain tissue than controls. TAC in plasma, SOD in erythrocytes, and GST in brain tissue homogenate were lower in Pb-group versus control. GTE co-administrated with Pb-reduced Pb contents, increased antioxidant status than Pb-group. In erythrocytes, Pb correlated positively with LPO and negatively with NO, GSH, SOD, and Hb. In brain tissue homogenate, Pb correlated positively with LPO and negatively with GSH. This study suggests that lead induce toxicity by interfering balance between prooxidant/antioxidant. Treatment of rats with GTE combined with Pb enhances antioxidant/ detoxification system which reduced oxidative stress. These observations suggest that GTE is a potential complementary agent in treatment of chronic lead intoxication.

Keywords Rat · Brain tissue homogenate · Erythrocyte · Lead toxicity · Green tea extract · Oxidative stress

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Introduction

Lead (Pb) is a heavy metal with no known biological functions in humans. It is naturally present in the lithosphere in negligible quantity, due to rock wind erosion or volcanic rejections. Nowadays, Pb is being a ubiquitous environmental contaminant due to its significant role in modern industry [37]. Effectively, Pb constitutes the most abundant non-essential element in the human organism due to its dispersion in ambient air, in many foods, in drinking water, and in

dust. Its toxicity is closely related to its accumulation in certain tissues, and its interference with the bioelements, whose role is critical for several physiological processes. Pb can damage various systems of the body including the renal, hepatic, hematological, and skeletal systems with the central nervous system being its primary target [33, 40].

It had been proposed that the hematological system is an important target of Pb-induced toxicity. Previous studies suggested that red blood cells (RBCs) have a high affinity for Pb. About 99% of the lead present in the blood is bound to erythrocytes, which makes them more vulnerable to oxidative damage than many other cells [20]. The neurotoxic effect of lead is a matter of serious concern. Behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and experimental animals have been recorded with lead blood (PbB) levels as low as 10 ug/dL [6]. Brain and neural tissues are highly sensitive to free radicals attack because neurons have an elevated metabolic rate and high content of oxidizable substrates. Additionally, the brain contains high concentrations of iron and has relatively weak antioxidant system as defense mechanisms except superoxide dismutase (SOD) [35].

Biochemical and molecular mechanisms of Pb toxicity are poorly understood. Various mechanisms were suggested to explain them: inhibition of the calcium-pump, a transport protein, disturbances in mineral metabolism, inactivation of several enzymes, demyelinization of nervous tissues, etc. [37]. Oxidative stress has also been proposed as another possible mechanism involved in lead toxicity. Lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) and reducing the antioxidant cell defense systems [32].

The deleterious consequences of membrane peroxidation have stimulated investigations on the efficacy and mechanisms of action of biologically relevant antioxidants, particularly naturally occurring ones, including those used as foods and beverages (i.e., green tea). The chemical composition of green tea extract (GTE) contains many polyphenolic compounds, generally known as catechins. The main catechins in green tea are (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate [7]. They are considered effective scavengers of superoxide, hydroxyl, and peroxyl radicals. A free radical–scavenging mechanism has generally been described as the basis of their antioxidative activity. In addition, catechins are known to chelate metal ions, another possible contributory mechanism in their protective action against the destructive effect of free radicals on the components of the cells [18].

This study was carried out to investigate the effects of chronic (6 weeks) oral administration of lead acetate (0.4%) on oxidative stress parameters in rat blood and brain tissue homogenate. Antioxidative activity of GTE (1.5%) against oxidative stress induced by chronic Pb administration was also evaluated by measuring plasma, erythrocytes, and brain tissue homogenate levels of prooxidant/antioxidant parameters [lipid peroxides (LPO), nitric oxide (NO), total antioxidant capacity (TAC), glutathione (GSH), and enzymes activities of SOD and glutathione-*S*-transferase (GST)]. Correlation between Pb levels and measured parameters was also evaluated.

Materials and methods

Chemicals

Thiobarbituric acid, butylated hydroxytoluene, reduced glutathione, sodium sulfate, sodium nitrite, epinephrine, lead acetate, naphthylethylenediamine dihydrochloride, sulphanilamide, 5′,5′-dithiobis-2-nitrobenzoic acid, and 1-chloro-2,4 dinitrobenzene were purchased from Sigma [St. Louis, MO, USA]. All other chemicals used were of analytical grade.

Animals

Sixty healthy adult male Sprague-Dawley rats [weight, 170–200 g; age, 3–4 months] were purchased from Animal House, Faculty of Medicine, Assiut University, Assiut, Egypt. The Ethical Committee of Assiut University approved the study. The animals were housed in clean plastic cages and allowed to acclimatize in the laboratory environment for a week [temperature 25°C, 12-h dark/light cycle]. In the experimental period, animals had access to food and water ad libitum. Green tea [*Camellia sinensis* (Linnaeus) O. Kuntze (standard research blends–lyophilized extract)] was provided by TJ Lipton [Englewood Cliffs, NJ]. GTE was made according to Maity et al. [27], by soaking 15 g of

instant green tea powder in 1 L of boiling distilled water for 5 min. The solution was filtered to make 1.5% and used as water extract.

Experimental design

The animals were divided into four groups, designated as control, green tea (GTE), Pb, and Pb+GTE. Animals in control group (n=15) drank distilled water as sole drinking source for 6 weeks. Animals in GTE-group (n=15) drank water containing GTE (15 g/L) for 6 weeks. Animals in Pb-group (n=15) drank water containing Pb (0.4% lead acetate) for same period [41]. Animals in Pb+GTE-group (n=15) drank water containing Pb (0.4% lead acetate and GTE 15 g/L) for same period. Twenty-four hours after last dose of treatment, all rats were killed under ether anesthesia by exsanguination. This level of anesthesia was sufficient for cardiac puncture and euthanasia as we did not observe any pain reflexes elicited by paw pinch. Blood was taken by cardiac puncture. The brain was removed and processed immediately for biochemical investigation, and the rest of brain was stored at −20°C for wet digestion in order to estimate of Pb^{2+}content.

Blood and tissues preparations

The blood was collected in two heparinized tubes. The first tube was centrifuged at 5,000 rpm for 10 min for plasma separation. The plasma sample was divided into aliquots and kept at −20°C until biochemical analyses. Erythrocytic lysate was prepared by washing RBCs after plasma separation with saline several times, and then RBCs were hemolyzed by addition of distilled water. The hemolysates were stored at −70°C until use. The second tube contained whole blood was used for Pb determination.

The brain was divided into two parts. The first part was homogenized in ice-cold 100 mM phosphate buffer (pH 7.4) using Potter–Elvehjem homogenizer fitted with a Teflon plunger. Homogenates were centrifuged at 11,000g for 20 min, and resulting supernatants were divided into aliquots and stored at –70°C. The second part of the brain was used for Pb determination as follows: brain tissue samples were carefully weighed (1 g), placed in polypropylene tubes, and digested in 1 ml of concentrated HNO_3 Suprapur (E. Merck) in a shaking water bath at 60°C for 30 min. This treatment ensures complete destruction of organic matter [10]. After digestion, a 100-µl aliquot was taken from clear solution and diluted (1:5 v/v) with deionized water. Lead calibration curves were constructed by adding known amounts of lead standard (E. Merck).

Biochemical assays

For measurement of lead in whole blood and brain tissue homogenates, analysis of diluted samples of whole blood and digested brain tissue were injected into atomic absorption spectrophotometer (Perkin–Elmer Model 400, Shelton, CT, USA) as previously described [47]. Hollow cathode lamps of Pb were used at wavelength of 283.3 nm. The results of Pb concentration in blood and brain tissue homogenates were expressed as part per million (ppm). Blood hemoglobin (Hb) was estimated using commercial kit (Randox Lab., Ltd., UK). Total proteins levels in brain tissue homogenates were determined chemically [26]. LPO levels were measured as thiobarbituric acid reactivities. The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described previously [45]. NO level was determined as total nitrite after deproteinzation with $ZnSO_4$ (30%), and reduction by cadmium in presence of NH_4Cl and sodium borate and color developed by reaction with Griess reagent was recorded at 550 nm against reagent blank using sodium nitrite 10–100 uM as standard [46]. SOD activity was determined according to its ability to inhibit auto-oxidation of epinephrine at alkaline medium [29]. GSH concentration was determined chemically as described by Dutta et al. [14]. GST activity was chemically determined using 1-chloro-2,4-dinitrobenzene substrate [21]. The plasma level of TAC (Biodiagnostic, Giza, Egypt) was measured by specific ELISA assay kit according to manufacturer protocol.

Statistical analysis

The results were expressed as mean±standard deviation (SD). Differences between groups were assessed by one-way analysis of variance with Tukey's test for multiple comparisons using SPSS software package for windows version 10. Correlation between lead and measured parameters was done using Pearson test. The level of significance was accepted with $P<0.05$.

Results

Plasma levels of LPO were higher ($P<0.001$) while NO, TAC, GSH, and SOD were lower ($P<0.001$, $P<0.001$, $P<0.001$, and $P<0.012$) in Pb-group than controls. In the GTE+Pb-group, levels of LPO was higher ($P<0.005$) while NO was lower ($P<0.001$) than in controls. Meanwhile, LPO was lower ($P<0.001$); TAC, GSH, and SOD were higher ($P<0.001$, $P<0.001$, and $P<0.002$) in GTE+Pb-group than Pb-group (Table 1).

Whole blood levels of Pb and erythrocytic levels of LPO were higher ($P<0.001$ for both), while erythrocytic levels of Hb, NO, SOD, and GSH were lower ($P<0.001$ for all) in Pb-group than controls. In GTE+Pb-group, levels of Pb were higher ($P<0.001$), but those of Hb, NO, and GSH were lower ($P<0.028$, $P<0.001$, and $P<0.001$) than in controls; meanwhile, Pb and LPO were lower ($P<0.016$ and $P<0.001$); Hb, SOD, and GSH were higher ($P<0.002$, $P<0.003$, and $P<0.001$) than Pb-group (Table 2).

Brain tissue levels of Pb and LPO were higher ($P<0.001$ for both), while levels of NO, GSH, and GST were lower ($P<0.001$ for all) in Pb-group than controls. In the GTE+Pb-group, levels of Pb were higher ($P<0.001$) while those of NO and SOD were lower ($P<0.001$ for both) than in controls; Pb and LPO were lower ($P<0.001$ for both); and those of SOD, GSH, and GST were higher ($P<0.001$, $P<0.001$, and $P<0.006$) than Pb-group (Table 3).

Whole blood Pb showed a significant positive correlation with erythrocytic levels of LPO ($r=0.678$, $P<0.001$) and negative correlation with erythrocytic levels of NO ($r=-0.697$, $P<0.001$), GSH ($r=-0.704$, $P<0.001$), SOD ($r=-0.333$, $P<0.009$), Hb ($r=-0.469$, $P<0.001$) of erythrocytes. While in the brain tissue homogenates, Pb showed positive correlation with LPO ($r=0.455$, $P<0.001$) and negative correlation with GSH ($r=-0.402$, $P<0.001$; Table 4).

Discussion

An adverse effect of lead on various human tissues was widely reported. Oxidative damage associated with the presence of Pb has been proposed to indicate a possible role of free radicals in the pathogenesis of lead toxicity [1]. In the current study, chronic oral drinking water containing 0.4% lead acetate for 6 weeks raised the whole blood and brain tissue homogenate levels of Pb and declined in Hb contents in Pb-treated rats. Similarly, Berrahal et al. [4] reported decrease in Hb levels in experimental rats exposed to two different doses of Pb [5 and 15 mg Pb^{2+}/kg body weight (b.w.) i.p. for 7 days]. On the contrary, Rao et al. [34] did not show any significant change in Hb level in battery workers [PbB 86.98 (SD 38.86)mg/dL]. Pb is known to interfere with heme and hemoglobin synthesis and also affect

Table 1 Plasma levels (mean±SD) of oxidative stress indices in different studied groups

Variables	Controls ($n=15$)	GTE-group ($n=15$)	Pb-group ($n=15$)	GTE+Pb-group ($n=15$)
LPO (μmol/dL)	0.550±0.117	0.619±0.110	1.324±0.447	0.851±0.300
Significance			$P<0.001$	$P<0.005$
				$P<0.001$[a]
NO (nmol/mL)	16.690±3.068	11.210±3.444	10.230±1.099	11.590±2.667
Significance			$P<0.001$	$P<0.001$
				$P>0.175$[a]
TAC (mmol/L)	1.686±0.297	1.780±0.302	0.925±0.235	1.518±0.235
Significance			$P<0.001$	$P>0.346$
				$P<0.001$[a]
GSH (μmol/dL)	1.636±0.526	1.391±0.189	0.8168±0.246	1.360±0.334
Significance			$P<0.001$	$P>0.249$
				$P<0.001$[a]
SOD (U/mL)	20.850±2.255	23.940±2.742	18.690±2.259	21.420±1.638
Significance			$P<0.012$	$P>0.429$
				$P<0.002$[a]

[a] *P* significance versus Pb-group

P significance versus control, *GTE* green tea extract, *Pb* lead, *LPO* lipid peroxide, *NO* nitric oxide, *TAC* total antioxidant capacity, *GSH* glutathione, *SOD* superoxide dismutase

Table 2 Whole blood lead and erythrocytic levels (mean±SD) of hemoglobin and measured oxidative stress parameters in different studied groups

Variables	Controls (n=15)	GTE-group (n=15)	Pb-group (n=15)	GTE+Pb-group (n=15)
Pb (ppm)	0.071±0.014	0.084±0.027	0.773±0.187	0.654±0.124
Significance			$P<0.001$	$P<0.001$
				$P<0.016$[a]
Hb (g/dL)	1.845±0.407	1.676±0.460	1.119±0.226	1.550±0.283
Significance			$P<0.001$	$P<0.028$
				$P<0.002$[a]
LPO (μmol/g Hb)	2.595±0.598	2.299±0.771	5.815±0.910	3.211±0.156
Significance			$P<0.001$	$P>0.105$
				$P<0.001$[a]
NO (μmol/g Hb)	90.780±29.044	70.380±14.935	43.420±6.356	39.280±6.179
Significance			$P<0.001$	$P<0.001$
				$P>0.506$[a]
GSH (μmol/g Hb)	9.572±2.160	8.528±1.747	2.580±0.931	6.541±2.388
Significance			$P<0.001$	$P<0.001$
				$P<0.001$[a]
SOD (U/g Hb)	0.445±0.136	0.365±0.140	0.239±0.075	0.391±0.169
Significance			$P<0.001$	$P>0.275$
				$P<0.003$[a]

[a] P significance versus Pb-group

P significance versus control, *GTE* green tea extract, *Pb* lead, *Hb* hemoglobin, *LPO* lipid peroxide, *NO* nitric oxide, *GSH* glutathione, *SOD* superoxide dismutase, *ppm* part per million

erythrocyte morphology and survival leading to anemia [24]. Pb stimulates iron-initiated membrane lipid oxidation by inducing changes in membrane physical properties [2]. It decreases life span of RBCs by inhibiting sodium–potassium ATPase and pyrimidine 5' nucleotidase, which impairs RBCs membrane stability by altering energy metabolism [31]. It decreases heme synthesis by inhibiting some of heme

Table 3 Brain tissue homogenate levels (mean±SD) of lead and oxidative stress indices in different studied groups

Variables	Controls (n=15)	GTE-group (n=15)	Pb-group (n=15)	GTE+Pb-group (n=15)
Pb (ppm)	0.5357±0.178	0.6732±0.221	1.927±0.769	1.207±0.611
Significance			$P<0.001$	$P<0.001$
				$P<0.001$[a]
LPO (nmol/mg protein)	1.857±0.353	1.690±0.320	2.801±0.711	2.182±0.269
Significance			$P<0.001$	$P>0.053$
				$P<0.001$[a]
NO (nmol/mg protein)	0.380±0.0151	0.307±0.118	0.209±0.091	0.228±0.075
Significance			$P<0.001$	$P<0.001$
				$P>0.635$[a]
GSH (nmol/mg protein)	11.710±1.345	13.260±1.790	8.958±1.747	12.060±1.509
Significance			$P<0.001$	$P>0.550$
				$P<0.001$[a]
GST (mM/min/g protein)	56.380±6.200	53.700±3.144	50.04±6.150	55.45±4.702
Significance			$P<0.001$	$P>0.625$
				$P<0.006$[a]
SOD (mU/mg protein)	2.289±0.305	3.399±1.293	1.905±0.529	3.309±0.767
Significance			$P>0.200$	$P<0.001$
				$P<0.001$[a]

[a] P significance versus Pb-group

P significance versus control, *GTE* green tea extract, *Pb* lead, *LPO* lipid peroxide, *NO* nitric oxide, *GSH* glutathione, *GST* glutathione-*S*-transferase, *SOD* superoxide dismutase, *ppm* part per million

Table 4 Correlation between lead in whole blood and brain tissue homogenate and oxidative stress indices in erythrocytic lysate and brain tissue homogenate

Parameters	Pb	
	Erythrocytic lysate	Brain tissue homogenate
Hb	−0.469, $P<0.001$	–
LPO	0.678, $P<0.001$	0.455, $P<0.001$
NO	−0.697, $P<0.001$	−0.186, $P<0.156$
GSH	−0.704, $P<0.001$	−0.402, $P<0.001$
GST	–	−0.242, $P<0.063$
SOD	−0.333, $P<0.009$	−0.242, $P<0.062$

Pb lead, *Hb* hemoglobin, *LPO* lipid peroxide, *NO* nitric oxide, *GSH* glutathione, *SOD* superoxide dismutase, *GST* glutathione-*S*-transferase, *SOD* superoxide

biosynthetic key enzymes pathway such as delta-aminolevulinic acid dehydratase (δ-ALAD), ferrochelatase, and coproporphyrinogen oxidase [16]. Furthermore, Pb interferes with iron utilization for heme formation in mitochondria, and radio-iron studies showed that lead competes with iron for its incorporation into RBCs [24]. A negative correlation between whole blood Pb and Hb levels in erythrocytes was reported in this study. This correlation is expected as lead interferes with Hb synthesis.

Confirming the previous studies, we found increased LPO in plasma, erythrocytic lysate, and brain tissue homogenates in Pb-treated rats. In consistence, Daniel et al. [12] reported increased LPO levels in brain tissue homogenates in rats that received lead [25 mg/kg i.p. for 7 days]. Bennet et al. [3] reported that (500 ppm) of lead acetate treatment in rat causes an increase in antioxidant enzymes and lipid peroxidation products in different brain regions. Meanwhile, Rao et al. [34] did not observe any significant change in blood lipid peroxidation in Pb-exposed battery workers [PbB 86.98 (SD 38.86)mg/dL]. A significant positive correlation was found in current study between Pb levels in whole blood and brain tissue homogenate and LPO. Previous studies on experimental animals had reported that Pb altered lipid metabolism and enhanced lipid peroxidation directly and indirectly. Gurer et al. [20] reported that increased fluxes of superoxide and H_2O_2 induce lipid peroxidation and oxidative stress, and these actions could be relevant to Pb toxicity. An inhibition of δ-ALAD activity leads to accumulation of δ-ALA, which undergoes autooxidation inducing free radicals and so lipid peroxidation [16]. In addition, Pb indirectly influences lipid peroxidation through damage the protective antioxidant barrier [33].

Nitric oxide is a lipophilic and chemically unstable molecule. It is a gaseous substance produced by nitric oxide synthase (NOS) from L-arginine. In the present study, levels of NO were significantly lower in plasma, RBCs, and brain tissue homogenates in Pb-treated rats than controls. Also, a negative correlation between Pb and NO levels in RBCs and brain tissue homogenates was observed. Similarly, Carmignani et al. [8] reported a significant reduction of plasma NO levels in rats exposed to lead acetate [60 ppm] in drinking water for 10 months. Pb affects NO production through inhibition of NOS activity [52] which might account for Pb neurotoxicity. Nitric oxide is primarily produced by neuronal nitric oxide synthase (nNOS) in response to activation of *N*-methyl-D-aspartate receptor (NMDAR), a class of glutamatergic receptors. Several studies show that calcium influx through NMDAR triggers nNOS activation. Because Pb can compete with calcium at NMDAR level, it could interfere with nNOS activation and consequent NO production [17].

Usually, the deleterious effects of oxidative stress are counteracted by natural defense mechanisms that involve enzymes and non-enzymatic scavengers of free radicals. GSH is one of the most important compounds, which helps in detoxification and excretion of heavy metals [43]. GSH can act as a non-enzymatic antioxidant by direct interaction of SH group with ROS or its involvement in enzymatic detoxification reactions for ROS as a cofactor [13]. In the current study, GSH levels were reduced in plasma, erythrocytic lysate, and brain tissue homogenates in Pb-treated rats than controls. Also, negative correlation was found between concentrations of Pb and GSH in erythrocytes and brain tissue homogenates. The decreased GSH levels after exposure to Pb observed in this study may result from high affinity of this metal to SH groups [19]. In this respect, Sugawara et al. [44] had reported a significant decrease in GSH content of erythrocytes from workers exposed to Pb [PbB 57.1 (SD 17.6)pg/dL]. Saxena and Flora [36] found that Pb exposure led to a pronounced depletion of rat brain GSH contents and these biochemical changes were correlated with increased uptake of Pb in blood and soft tissues. On the contrary, Patra et al. [33] found no significant

changes in total thiol levels in brain of Pb-treated rats [1 mg of Pb^{2+}/kg b.w i.p. for 4 weeks]. Meanwhile, Rao et al. [34] found marked increase in blood GSH level in Pb toxicity [PbB 86.98 (SD 38.86)mg/dL].

It has been revealed that Pb may affect antioxidant barrier via inhibiting activities of enzymes involved in GSH metabolism, such as GST and SOD by blocking their SH groups [19]. In this study, GST activity in brain tissue was decreased in Pb-group compared with control. Hunaiti and Soud [22] showed that the activities of GST, glutathione peroxidase, and reductase, as well as the content of blood GSH, had decreased with an increasing lead concentration in the blood that reached 50% inhibition at a lead salt concentration [6,000 μg/dL] in occupational workers exposed to this metal. On the contrary, Bokara et al. [5] reported that GST activity increased with Pb exposure time in rat's brain tissues that drank lead acetate [500 ppm for 8 weeks], showing protection against Pb acetate toxicity.

SOD is a metalloprotein and accomplishes its antioxidant functions by enzymatically detoxifying the superoxide anion. SOD dismutates superoxide into H_2O_2 and needs copper and zinc for its activity. In this study, SOD activities in plasma and RBCs were significantly decreased in Pb-group compared with control. Meanwhile, SOD activity of the brain tissue homogenates were not significantly reduced. The lower activities of SOD obtained in this study may be partly explained by interaction between Pb and essential metals such as copper and zinc in plasma and erythrocytes that are essential cofactors for SOD [32]. Another possible explanation is the massive production of superoxide anions, which overrides enzymatic activity and so leads to fall in SOD concentration. Consistent with others [34], a negative correlation was found in this study between whole blood Pb and SOD concentrations in erythrocytes. Various reports regarding influence of Pb on SOD activities have given divergent results. Ito et al. [23] observed a drop of SOD activity in blood of people with [PbB 30–40 μg/dL], whereas SOD activity in blood plasma was unchanged. Sugawara et al. [44] noticed a lower activity of erythrocyte's SOD of 57% [PbB 57.1 μg/dL], but simultaneously noted a raised activity of blood plasma SOD of 80%. Berrahal et al. [4] noticed decreased SOD activity in rat's erythrocytes only after exposure to high dose of lead [15 mg Pb/kg b.w.]. Mohammad et al. [30] noticed decreased in plasma SOD activity in painters with blood lead levels [≤400 μg/L]. In consistence, Patra et al. [33] found no significant alteration in SOD activity in rat's brain exposed to Pb acetate [1 mg/kg b.w., i.p. for 4 weeks]. In contrary, Costa et al. [11] observed over three times higher activity of SOD in blood of people with [PbB 53.4 μg/dL] and also a strong, positive correlation between SOD activity and PbB. Ye et al. [49] observed higher activity of SOD in blood of people with [PbB>37 μg/dL]. Meanwhile, Wasowicz et al. [48] did not observe any changes in SOD activity in erythrocytes in people with [PbB 0.4 μg/dL] compared with control. Yin et al. [50] reported decreased SOD activity in brain tissue of rats' drinking water contained 0.2% lead acetate for 23 days. Confirming that antioxidants are affecting by chronic lead administration, this study showed decreased of plasma level of TAC in Pb-treated group than controls.

The potential role of oxidative stress injury, which is associated with Pb, suggests that antioxidants may enhance the efficacy of treatment designed to mitigate Pb-induced toxicity. In the current study, oral administration of GTE [1.5%] combined with Pb [0.4% lead acetate] for 6 weeks resulted in decrease in Pb concentration of whole blood and brain tissue homogenate. It also resulted in partial correction of anemia and raised Hb concentration, decreased lipid peroxidation process, as well as raised TAC, SOD, GSH, and GST activity in plasma, erythrocytes, and brain tissue homogenates. The decline in Pb levels in whole blood and brain tissue homogenates in the GTE +Pb-treated group can be explained by chelating property of catechins of GTE which can decrease Pb lipophilicity and so its absorption from gastrointestinal tract [28]. Compounds of green tea scavenge a wide range of free radicals which may initiate lipid peroxidation. GTE may chelate metal ions, especially iron and copper, which, in turn, inhibit the generation of hydroxyl radicals and degradation of lipid hydroperoxides resulting in reactive aldehydes formation [18]. GTE enhance RBCs resistance to oxidative stress in vitro and in vivo and thus can reduce anemia resulting from lead exposure [51]. Lee et al. [25] found that green tea polyphenol (−)-epigallocatechin gallate inhibits H_2O_2 or ferrous ion-induced lipid peroxidation in gerbil brain homogenates, and also inducible nitric oxide synthase and nNOS induction [9]. Meanwhile, Shin et al. [38] reported that GTE might play a crucial

role of NO inhibition as free radical scavenging effect rather than induced NOS inhibition. It had been reported that GTE decreased plasma NO level [39] and increased total plasma antioxidant activity [42]. Erba et al. [15] found that subjects drank two cups of green tea daily for 42 days; their endogenous plasma TAC was increased while their plasma peroxides and oxidative stress-induced damage were decreased. Also, Yin et al. [50] found that green tea could reverse oxidative stress which had been impaired in rats drinking water containing 0.2% lead acetate for 23 days.

Conclusion: alterations in several indicators of oxidative stress in this animal model of chronic lead administration suggested that cellular damage mediated by free radicals is involved in the pathophysiology associated to lead toxicity. This findings emphasis the importance of measurement of blood Pb concentrations in the general population to combat Pb toxicity effects before clinical signs predominate. The supplementation with GTE, an antioxidant and chelator, could recover these oxidative damages partly. Taking into consideration that metabolism of lead and catechins is the same in rats as in human beings, results obtained from the current study support the suggestion that green tea may also protect red blood cells and brain cells of human beings against sequelae of oxidative stress caused by Pb intoxication. Further investigations are warranted to better understand the underlying mechanisms for the beneficial effect of GTE, as well as its optimum dosage and duration in the clinical lead intoxication cases. Information on potential interactions between the constituents of green tea and lead will lead to a clearer and better understanding of the potential health effects of green tea.

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