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NEUROPROTECTIVE EFFECT OF GREEN TEA EXTRACT AGAINST LEAD TOXICITY IN RATS

(With 2 Tables and 8 Figures)

By

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التأثير العصبي الوقائي لخلاصة الشاي الأخضر ضد التسمم بالرصاص في الجرذان

السيد الديب مهني ، عبد الرحيم مكى

تعتبر سمية الرصاص مصدر قلق على الصحة العامة لانتشاره في البيئة. ويعتبر المخ من أكثر الأعضاء عرضة للتسمم به. وجد أن الشاي الاخضر يعمل كمضاد للأكسدة ومزيل للشقوق الطليقة وعامل خالب. أجريت هذه الدراسة لمعرفة تأثير الشاي الاخضر على الحد من سمية الرصاص على أمخاخ ذكور الجرذان. أشتملت هذه الدراسة على أربع مجموعات من ذكور الجرذان (15جرذ في كل مجموعة) قسمت على النحو التالي: المجموعة الضابطة، مجموعة أعطيت الشاي الاخضر (1.5%)، ومجموعة أعطيت الرصاص (0.4% خلات الرصاص في الماء المقطر)، مجموعة أعطيت الرصاص + الشاي الاخضر في مياه الشرب لمدة 6 أسابيع. وقد تم قياس مستويات بيروكسيد الدهون، اكسيد النتريك ، سعة مضادات الأكسدة الكلية، الجلوتاثيون، محول الجلوتاثيون -S، فوق اكسيد ديسموتاز في البلازما وكرات الدم الحمراء وأنسجة المخ باستخدام المقياس اللوني. وتم قياس مستويات الرصاص باستخدام مقياس طيف الامتصاص الذري . كانت مستويات الرصاص في المجموعة التي عولجت بالرصاص أعلى في الدم، أنسجة المخ من المجموعة الضابطة، وكانت مستويات بيروكسيد الدهون أعلى بينما كانت مستويات اكسيد النتريك وسعة مضادات الأكسدة الكلية والجلوتاثيون أقل في البلازما وكرات الدم الحمراء وأنسجة المخ من المجموعة الضابطة. كانت مستويات فوق اكسيد ديسموتاز في الكريات الحمراء ومحول الجلوتاثيون -S في انسجة المخ أقل في المجموعة التي عولجت بالرصاص من المجموعة الضابطة. يبدو ان اضافة الشاي الاخضرالي المجموعة التي عولجت بالرصاص أكثر فعالية في تقليل الرصاص وبيروكسيد الدهون، وزيادة مضادات الأكسدة في البلازما وكرات الدم الحمراء وانسجة المخ مقارنة بالمجموعة التي عولجت بالرصاص فقط. كان أيضا هناك علاقة ايجابية ذات دلالة احصائية بين الرصاص وبيروكسيد الدهون وعلاقة سلبية بين الرصاص والجلوتاثيون في كرات الدم الحمراء وأنسجة المخ. وفي الوقت نفسه، وجدت علاقة سلبية ذات دلالة احصائية بين الرصاص واكسيد النتريك وفوق اكسيد ديسموتاز والهيموجلوبين في كرات الدم الحمراء. تشير هذه الدراسة إلى أن الرصاص يمكن أن يحدث سميته عن طريق الأخلال على التوازن بين العوامل المساعدة و المضادة للأكسدة. اضافة الشاي الاخضر الي المجموعة التي عولجت

بالرصاص يمكن أن يعزز مضادات الأكسدة وإزالة ارصاص وبالتالي يقلل اجهاد الاكسدة وعبء الرصاص في المخ وخفض سميتهوأضراره بالأنسجة.

SUMMARY

The toxicity of lead (Pb) is of concern to public health due to its persistence in the environment. Brain is one of the major target organs where severe neurological alternations may be triggered after exposure. Pb could disrupt prooxidant/antioxidant balance of tissue which leads to physiological dysfunction. Green tea extract (GTE) is antioxidant, free radicals scavenger and has chelating property. This study was conducted to investigate effect of GTE on reducing Pb toxicity of the brain of male rats. Four groups of male rats (each 15 rats) were utilized as following: control, GTE–group (1.5% w/v), Pb–group (0.4% lead acetate in distilled water), Pb + GTE-group. Rats received GTE and/or lead orally in drinking water for 6 weeks. Levels of oxidant and antioxidant [lipid peroxides (LPO), nitric oxides (NO), total antioxidant capacity (TAC), glutathione (GSH), glutathione S-transferase (GST), superoxide dismutase (SOD)] were measured using colorimetric methods. Pb concentration in brain tissue was measured by atomic absorption spectrometer. Histological sections of brain tissues were prepared and examined using the routine pathological technique. Pb concentrations in Pb-treated group were higher in brain tissue than controls. In Pb-group, levels of LPO were higher while, NO and GSH were lower in plasma than controls. Plasma level of TAC was lower in Pb- treated group than control. Levels of SOD; GST in tissue were lower in Pb-exposed rats versus control. GTE co-administrated with Pb appeared more effective in reduction of Pb contents, LPO and increase antioxidant status in plasma and brain tissue comparing to Pb-group. Also, severe destructive changes were observed in the brain tissue, treated with Pb alone, represented by meningitis, neuronal degeneration, cerebral infarction, interphase encephalitis and purkinje cell layer degeneration and necrosis in the cerebellum. While the cases treated with lead and GTE, showed marked improvement in the cellular structure of the brain, represented by vacuolated neurons and absence of necrosis of the cerebellar purkinje cell layer. The data from this study suggest that lead can induce toxicity by interfering with delicate balance between pro- and antioxidants. The treatment of rats with GTE combined with Pb could enhance antioxidant/ detoxification system which consequently reduced oxidative stress and Pb burden in the brain thus potentially reducing Pb toxicity

and tissue damage. Running title: Lead toxicity: Effect of Green Tea Extract on Oxidative Stress in Rat Brain.

Keywords: *Rat, brain, lead toxicity, green tea extract, oxidative stress.*

INTRODUCTION

Lead (Pb) is an environmental and industrial pollutant that has been detected in every facet of environmental and biological systems. Pb can be found in water pipes, insecticides, lining of equipment where corrosion resistance and pliability are required, in petroleum refining, in construction, x-ray and atomic radiation protection and is a major industrial byproduct. The manipulation of Pb for these uses has caused Pb contamination of air, dust, and soil. Pb poisoning is considered to be one of the most difficult environmental health problems, since it does not show any unique manifestation during its early stage (Patrick, 2006). It has been found to produce wide range of biochemical and physiological dysfunctions in humans and animals (Courtois *et al.*, 2003).

Lead toxicity is related to haemopoietic, renal, nervous, gastrointestinal and reproductive disorders in man and animals. (Taieb *et al.*, 2006). Abbas *et al.* (2003) studied the toxic effects of lead acetate (0.01%, 0.05% and 0.1% in drinking water for 30 days) in male albino rats. The brain lesion consisted of demyelinization and collagenous scar formation with neuronal atrophy in hippocampus due to free radical production and the Pb induced oxidative stress. Also neuronal degeneration of the cerebellum, disruption of the normal arrangement of cell layers (Cerebellum) and large spaces showed in between purkinje cell layer and the cerebellum granular layer, detected in male albino rats exposed to 50 mg /kg Bw of lead acetate for 8 weeks (Pardeep and Bimla, 2004).

Lead intoxication affects the nervous system causing peripheral neuropathy in adults and encephalopathy in children. The most important symptoms of pediatric lead poisoning are drowsiness, irritability, vomiting, gastrointestinal symptoms, ataxia, stupor and fatigue (Busselberg *et al.*, 1993). Pathological findings due to lead toxicity include gout, hypertension, sterility, spontaneous abortions, neonatal mortality and morbidity**.** (Ferguson, 1990).

Although several mechanisms have been proposed to explain the Pb-induced toxicity, no mechanisms have been yet defined explicitly. One of the proposed mechanisms is that Pb-induced oxidative stress

contributes to the deleterious effects by disrupting the delicate prooxidant/antioxidant balance that exists within mammalian cells (Adonaylo and Oteiza 1999). Hydroxyl radicals generated from hydrogen peroxide and ferrous ions which are produced *in vivo*, may initiate and propagate the degenerative reaction in the cell membranes known as lipid peroxidation (Halliwell, 1994).

It has been also reported that Pb exposure has a dose response relationship with changes in antioxidant enzyme levels and their activities as superoxide dismutase (SOD), catalase, and glutathione-S-transferase (GST). GST is cytosolic enzyme involved in detoxification of a range of xenobiotic compounds by conjugation to glutathione (GSH) and also provides protection against oxidative stress (Adonaylo and Oteiza 1999; Annabi *et al.,* 2007). Also, the pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins, alters calcium homeostasis, and lowering the level of available sulfhydryl antioxidant reserves in the body. The propensity for lead to catalyze oxidative reactions and generate reactive oxygen species has been demonstrated. These Reactive Oxygen Species (ROS) inhibit the production of sulfhydryl antioxidants, inhibit enzyme reactions impairing heme production, cause inflammation in vascular endothelial cells, damage nucleic acid, inhibit DNA riper, and initiate lipid peroxidation in cellular membranes. (Patrick, 2006).

Herbal medicines derived from plant extracts being increasingly utilized to treat wide variety of clinical disease. Green tea (GT) is reported to delay or prevent certain forms of cancer, arthritis, cardiovascular and other disorders. An antioxidant is a molecule capable of preventing the oxidation of other molecules. As a result, antioxidants are often reducing agents such as polyphenols (Halliwell, 2008). Green tea is rich in catechins i.e., polyphenolic compounds whose antioxidant - oxidant activity is severalfold higher than the vitamin C and E. Catechins can prevent lipid hydroperoxide formation and toxicity. Scavenge superoxide and other free radicals and peroxytrite, all of which have been implicated in diabetes complications. Catechins were also shown to alter the catalytic activity of oxidative enzymes and chelate iron and copper, thus preventing metal-catalyzed free-radicals formation and the later has been associated with neuropathy in diabetic rats (Georgian *et al.,* 2005). The therapeutic potential of green tea also, due to antioxidant activity of catechins, which binds with metal ions to form insoluble complex –ionic salt used to remove the lead metal. Catechins

also inhibits the arachidonic acid cascade and normalizes bone metabolic disorders in lead –poisoned rats. (Dina, 2008). The Green Tea has antioxidant, hepatoprotective, chemo protective and anticarcinogenic effects (Nakagawa and Yokozawa, 2002).

Green tea has also a highly reputed chemotherapeutic effects and is one of the most widely investigated herbs. Since it has been imbibed in China, Korea and Japan for thousands of years, its long-term safety is well established. The most important constituent of green tea is catechins, which is characterized by its ability to scavenge free radicals from damaging biomolecules (antioxidant) and quench singlet oxygen from activating organic molecules to form peroxidase and free radicals. Such properties prevent DNA damages by reactive oxygen species. Catechins are therefore both anti-mutagenic and anti-carcinogenic. (Aysebelin *et al.,* 2008).

The aim of the present study was conducted to investigate protective effect of GTE in reducing lead toxicity on brain of male albino rats.

MATERIALS and METHODS

I- Chemicals:

Thiobarbituric acid, butylated hydroxytoluene, reduced glutathione, sodium sulphate, sodium nitrite, epinephrine, lead acetate, naphthylethylenediamine dihydrochloride, sulphanilamide, 5',5'-dithiobis-2-nitro-benzoic acid and 1 chloro-2,4 dinitrobenzene were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

II- Animals and Experiment:

Sixty healthy male Sprague-Dawley rats (170-200 gram) were purchased from Animal House, Faculty of Pharmacy, King Saud University, KSA. All animals were conditioned at room temperature at a natural photoperiod for one week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 6 weeks. The animals were randomly divided into 4 groups (15 rats each) as the following; Group I (Control group) received distilled water as sole drinking source. Group II (GTE group) received GTE (1.5% w/v). Group III (Pb group) received 0.4% lead acetate in distilled water (Sivaprasad *et al.,* 2004), Group IV (Pb + GTE group) received mixture of lead acetate and GTE. The solutions used in groups II, III, IV from beginning of experiment as

their sole source of drinking water. GTE was made according to Maity *et al.* (1998), by soaking 15 g of instant green tea powder in one liter of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% GTE.

III- Biochemical Analysis:

The animals of different groups were narcotized using ether and sacrificed one day after the end of treatment. The brain was excised immediately for biochemical parameters examinations. Brain was divided into two parts. One part was weighed (one gram) and digested for Pb determination. The second part was homogenized in ice-cold 100mM phosphate buffer (pH 7.4) using Potter-Elvehjem homogenizer fitted with a taflon Plunger. Homogenates were centrifuged at 11,000 R for 20min and resulting supernatants were divided into aliquots and stored at – 80 ^{o}C.

The blood sample from each rat was collected from orbital vein in two heparinized tubes. The first tube was centrifuged at 5000 rpm for 10 min for plasma separation. The plasma sample was divided into aliquots and kept at -20^{o}C until biochemical analyses.

Pb levels were determined in brain tissue. Brain tissue samples were carefully weighed, placed in polypropylene tubes, and digested in 1ml of concentrated HNO_3 in a shaking water bath at 60°C for 30min. This treatment ensures complete destruction of organic matter (Christian, 1969). After digestion, 100ul aliquot was taken from clear solution and diluted (1:5 v/v) with deionized water. Calibration curves were constructed by adding known amounts of lead standard

(E. Merck). Analysis of diluted samples of blood and digested tissue were injected into atomic absorption spectrophotometer (Perkin-Elmer Model 400, Shelton, CT, USA) as previously described (Villeda-Hernandez *et al.,* 2001). Hollow cathode lamps of Pb were used at wavelength of 283.3nm. The levels of LPO were measured as thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde and thiobarbituric acid was measured as described (Thayer, 1984). The levels of NO was determined as total nitrite after deproteinzation with $ZnSO_4$ (30%), and color developed by reaction with Griess reagent (1% sulfanilamide/ 0.1% naphthyelthylene diamine diHCL, w/v in 2.5% H_3PO_4) was recorded at 550nm against reagent blank using sodium nitrite 10-100 uM as standard (Ding *et al.,* 1988). GSH concentrations were determined chemically as described by Dutta *et al.* (1995). The plasma level of TAC (Biodiagnostic, Giza,

Egypt) was measured by specific ELISA assay kits according to manufacturer protocol. SOD activity was determined according to its ability to inhibit autooxidation of epinephrine at alkaline medium (Misra and Fridovich, 1972). GST activity was chemically determined using 1-chloro-2, 4-dinitrobenzene substrate (Habig *et al.*, 1973).

IV- Histological analysis*:*

After the end of the experiment the animals were narcotized using ether and sacrificed. Specimens from brain tissues were collected and fixed in 10 % neutral buffered formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casting, blocking, cutting at 2-5 µm thickness and stained using the routine pathological technique that used by (Bancroft, 1975).

V- Statistical analysis:

The results are expressed as mean±standard error (SE). Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the SPSS software package for windows version 10. Correlation between lead and measured parameter was done using Pearson test. The level of significance was accepted with $P \leq 0.05$. (Snedecor and Cochran, 1980).

RESULTS

Biochemical Results:

Plasma levels of NO was lower ($P < 0.001$) while, SOD was higher ($P < 0.01$) in GTE-group than control. LPO was higher ($P < 0.001$) while, TAC, GSH were lower ($P < 0.001$) in Pb- group than control and GTE-groups. Meanwhile, NO was lower than control ($P < 0.001$), while SOD was lower than GTE-group ($P < 0.001$) in Pb-group. In GTE+Pb-group, LPO was higher ($P < 0.05$), while NO was lower ($P < 0.001$) versus controls; SOD was higher ($P < 0.05$) versus GTE-group; LPO was lower ($P < 0.001$), TAC, GSH, SOD were higher ($P < 0.001$, $P < 0.001$, $P < 0.01$) versus Pb-group (Table 1).

Table 1: Plasma levels (mean±SEM) of oxidative stress indices in different treated groups.

Variables	Controls	GTE-group	Pb –group	GTE+Pb- group
LPO (µmol/dL)	0.550 ± 0.030	0.619 ± 0.028	1.324 ± 0.115	0.851 ± 0.077

Significance		P >0.05	P <0.001 *P <0.001	P <0.05 *P >0.05 #P <0.001
NO (nmol/ml)	16.690 ± 0.792	11.210± 0.889***	10.230 ± 0.283	11.590 ± 0.689
Significance		P <0.001	P <0.001 *P >0.05	P <0.001 *P >0.05 #P >0.05
TAC (mmol/L)	1.686 ± 0.077	1.780 ± 0.078	0.925 ± 0.061	1.518 ± 0.074
Significance		P >0.05	P <0.001 *P <0.001	P >0.05 *P >0.05 #P <0.001
GSH (µmol/dL)	1.636 ± 0.136	1.391 ± 0.049	0.8168 ± 0.064	1.360 ± 0.086
Significance		P >0.05	P <0.001 *P <0.001	P >0.05 *P >0.05 #P <0.001
SOD (U/ml)	20.850 ± 0.582	23.940 ± 0.708	18.690 ± 0.583	21.420 ± 0.423
Significance		P <0.01	P >0.05 *P <0.001	P >0.05 *P <0.05 #P <0.01

P: Significance versus control, *P: Significance versus GTE group, #P: Significance versus Pb- group. GTE: green tea extract; Pb: lead; LPO: lipid peroxide; NO: nitric oxide; TAC: total antioxidant capacity; GSH: glutathione; SOD: superoxide dismutase.

In the brain tissue, SOD was higher (P <0.01) in GTE-group than control. In Pb-group, Pb, LPO were higher (P <0.001) while GSH was lower (P <0.001) than control and GTE-group. Meanwhile, in Pb-group, lower levels were found of NO, GST (P <0.001, P <0.01) than control and of SOD than GTE-group (P <0.001). In GTE+Pb- group, Pb, SOD were higher (P <0.01, P <0.01) while, NO was lower (P <0.01) than controls. Pb, LPO were higher (P <0.05, P <0.05) than GTE-groups. Pb, LPO were higher (P <0.01, P <0.01) while, SOD, GSH, GST were lower (P <0.001, P <0.001, P <0.05) than Pb-group (Table 2).

Table 2: Brain tissue levels (mean±SEM) of lead and oxidative stress indices in different treated groups.

Variables	Controls	GTE-group	Pb -group	GTE+ Pb - group
Pb (ppm)	0.5357 ± 0.046	0.6732 ± 0.057	1.927 ± 0.199	1.207 ± 0.158
significance		P >0.05	P <0.001	P <0.01

			*P <0.001	*P <0.05 #P <0.01
LPO (nmol/ mg protein)	1.857 ± 0.091	1.690 ± 0.083	2.801 ± 0.184	2.182 ± 0.069
significance		P >0.05	P <0.001 *P <0.001	P >0.05 *P <0.05 #P <0.01
NO (nmol/ mg protein)	0.380 ± 0.039	0.307 ± 0.031	0.209 ± 0.024	0.228 ± 0.020
significance		P >0.05	P <0.001 *P >0.05	P <0.01 *P >0.05 #P >0.05
SOD (mU/ mg protein)	2.289 ± 0.079	3.399 ± 0.334	1.905 ± 0.137	3.309 ± 0.198
significance		P <0.01	P >0.05 *P <0.001	P <0.01 *P >0.05 #P <0.001
GSH (nmol/ mg protein)	11.710 ± 0.347	13.260 ± 0.462	8.958 ± 0.451	12.060 ± 0.390
significance		P >0.05	P <0.001 *P <0.001	P >0.05 *P >0.05 #P <0.001
GST (mM/ min/g protein)	56.380 ± 1.601	53.700 ± 0.812	50.04 ± 1.588	55.45 ± 1.214
significance		P >0.05	P <0.01 *P >0.05	P >0.05 *P >0.05 #P <0.05

P: Significance versus control, *P: Significance versus GTE group, #P: Significance versus Pb- group. GTE: green tea extract; Pb: lead; LPO: lipid peroxide; NO: nitric oxide; SOD: Superoxide dismutase; GSH: glutathione; GST: glutathione S- transferase.

Pathological Results:

The brain of rats, treated with lead acetate alone, showed meningeal hemorrhage, congestion and edema (Fig. 1).

Neuronal degeneration, atrophy, necrosis, central chromatolysis, neuronophagia and Satelletosis were also observed Fig. 2 (A&B).

Encephalitis, represented by, diffuse mononuclear cells infiltration, congestion and perivascular edema and cerebral infarction were also noticed (Fig. 3 A,B and C).

Degeneration as well as necrosis of the purkinje cell layer of the cerebellum showed (Fig. 4).

Disarrangement of the cell layers of the cerebellum with more than one space in between its granular layer and purkinje cell layer (Fig.5).

Marked improvement showed in the brain of cases treated with lead acetate and GTE represented by, no spaces showed in between the granular and the purkinje layer of the cerebellum, also no degeneration or necrosis showed in the purkinje cell layer' just vacuolated neurons showed (Fig. 6-8).

Neither characteristic pathological changes in the brain showed in cases treated with GTE alone nor control cases.

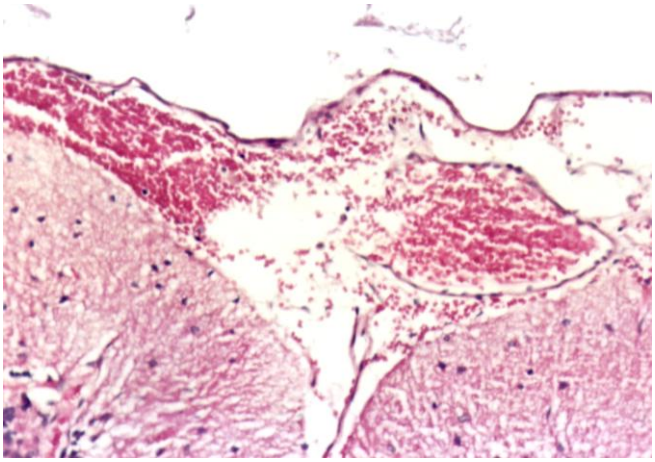


Fig. 1: Brain of male albino rat, intoxicated with lead acetate alone, Showing: Meningeal hemorrhage, edema and congestion. H&E. X. 200.

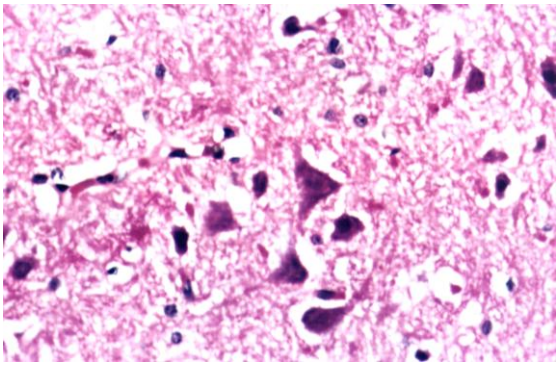
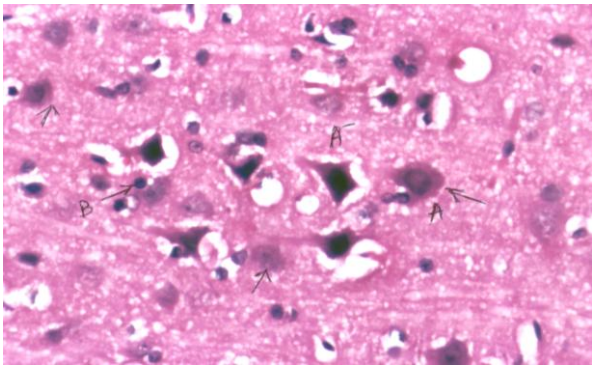


Fig. 2:

A- Brain of male albino rat, intoxicated with lead acetate alone, Showing: Central chromatolysis, neuronal degeneration, atrophy and neuronophagia. H&E.X. 400.



B- Brain of male albino rat, intoxicated with lead acetate alone, Showing: Neuronal degeneration (A), necrosis, edema and Satelletosis (B). H&E.X. 400.

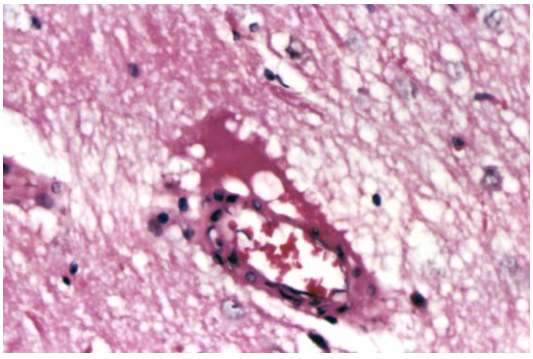


Fig. 3:

A- Brain of male albino rat, intoxicated with lead acetate alone, Showing: Congestion and edema H&E.X.400.

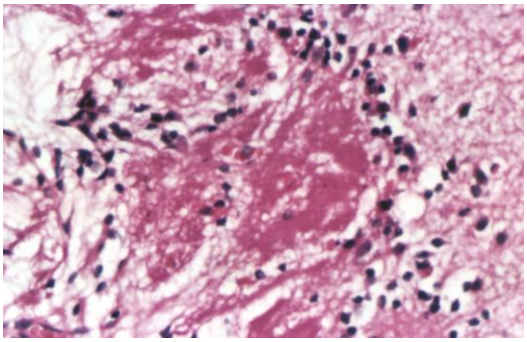
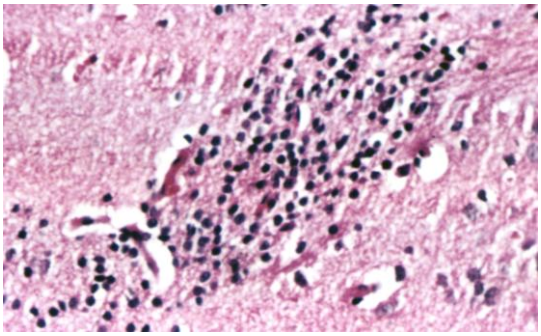


Fig. 3: B- Brain of male albino rat, intoxicated with lead acetate alone, Showing: Cerebral infarction with mononuclear cells infiltration. H&E.X. 400.



C- Brain of male albino rat, intoxicated with lead acetate alone, Showing: Diffuse Lymphocytic cells infiltration (Interface encephalitis). H&E.X.200.

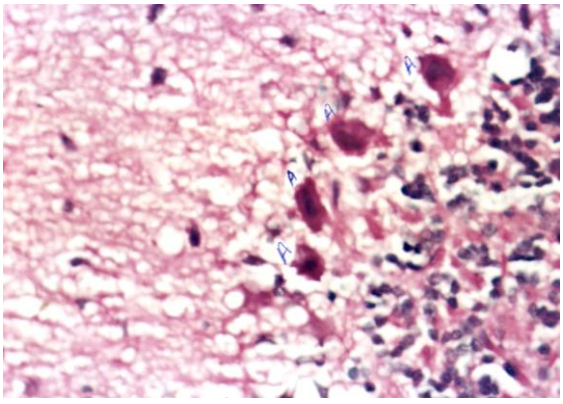


Fig. 4: Brain of male albino rat, intoxicated with lead acetate alone, Showing: Necrosis of the purkinje cell layer of the cerebellum (A). H&E.X. 400.

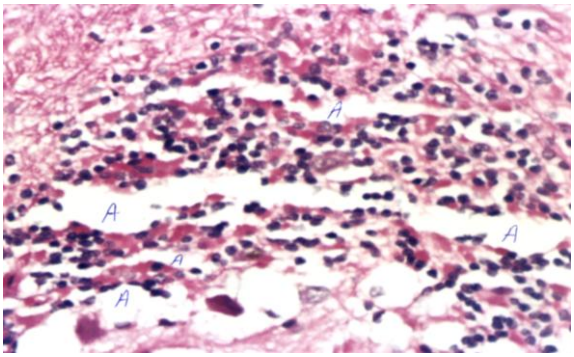
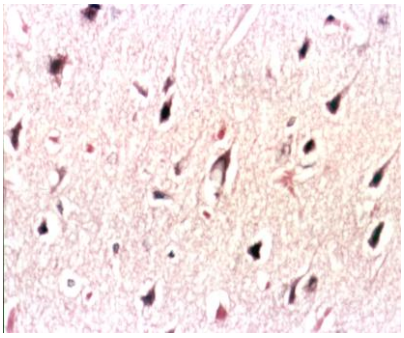


Fig. 5: Brain of male albino rat, intoxicated with lead acetate alone, Showing: Disruption of the normal arrangement of cell layers of cerebellum and presence of large spaces in between purkinje cell layer and granular layer (A). H&E.X.400.



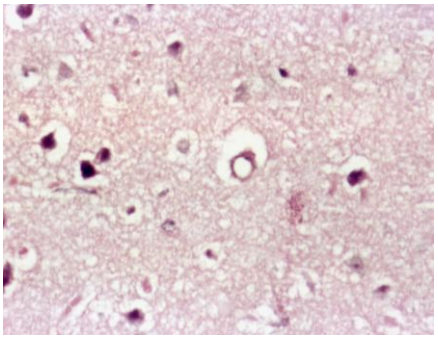


Fig. 6&7: Brain of male albino rat, intoxicated with lead acetate and GTE, Showing: Marked improvement "just vacuolated neurons". H&E.X. (200& 400).

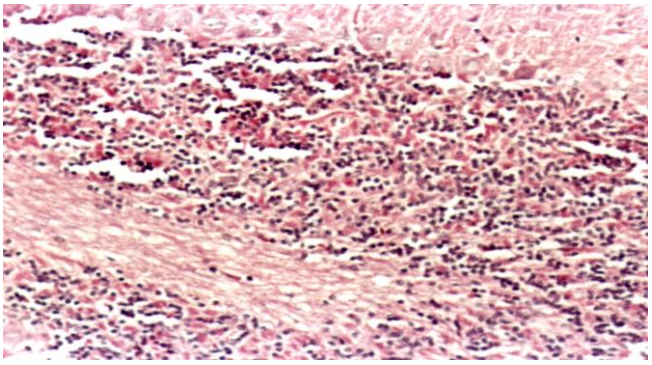


Fig. 8: Brain of male albino rat, intoxicated with lead acetate and GTE, Showing: Normal arrangement of the cerebellum layers and no spaces in between its granular and purkinje layers. H&E.X. 200.

DISCUSSION

Lead is a leading cause of human brain intoxication. Lead exposure at young age can hurt the brain's development and cause learning and behavioral problems such as attention, memory, learning, emotional and other behavioral problems that persist into adulthood. It may also interfere with recovery from a brain injury and alters the normal development of newly born neurons in a part of the brain (hippocampus) known to be important for learning and memory. (Guilarte, 2007; Schneider, 2009). An adverse effect of lead was widely reported in urban areas and usually associated with the outcomes of pregnant impairment, such as mental retardation, learning disabilities, low birth weight and hearing loss (West *et al.,* 1994).

In the present study, severe neurodegenerative changes showed in brain meanings, cerebrum and cerebellum, represented as edema, congestion and hemorrhage, neuronal degeneration, neuronophagia, satelletosis, gliosis, degeneration as well as necrosis of the purkinje cell layer of the cerebellum, disruption of its layers and many spaces showed between its layers. (Canfield *et al.,* 2003 and Mendola *et al.,* 2002) demonstrated that the toxic effects of the lead principally were manifested in the central nervous system leading to destruction of the blood brain barrier, which leads to edema, loss of neurons and reactive gliosis as showed in our study (Canfield *et al.,* 2003 and Mendola *et al.,* 2002).

Astrocytes were responsible for sequestration of lead in brain tissue and the activation of the astroglia may lead to loss of the buffering function and contribute to the pathological changes, such as neuronal cell death that usually accompanied with inflammatory cell infiltration and production of both cytokines and chemokines (Lidia *et al.,* 2006 and Thomas, 2007). Oxidative damage associated with the presence of Pb in the brain has been proposed to indicate a possible role of free radicals in the pathogenesis of lead toxicity (Adonaylo and Oteiza, 1999). The potential role of oxidative stress injury, which is associated with Pb poisoning, suggests that antioxidants may enhance the efficacy of treatment designed to mitigate Pb -induced toxicity. (Daggett *et al.,* 1998).

In consistence with other researchers, in this work the levels of Pb brain tissues were significantly higher in Pb treated group than controls (Patra *et al.,* 2001; Villeda-Hernandez *et al.,* 2001; Yin *et al.,* 2008). After combination of GTE with Pb, levels of Pb in brain tissue were significantly reduced comparing with Pb-treated group but still

significantly higher than controls that confirmed by the marked improvement showed in brain tissue histollogically, as no were evident spaces showed in the cerebellum, no necrosis of the purkinje cell layers, just vacuolated neurons and congestion. This can be explained by the chelating property of catechins of green tea which can decrease Pb lipophilicity, and thus its gastrointestinal tract absorption and leads to its chelation (Mandel *et al.*, 2006).

In addition, others reported that Pb is not able to induce free radicals directly, but it indirectly influences the processes of lipid peroxidation through damaging the protective antioxidant barrier (Patra *et al.*, 2001). In this study, the GTE combined with Pb showed significantly reduction of LPO levels return to control. The efficiency of green tea in preventing lipid peroxidation was revealed also by (Ostrowska *et al.*, 2004). Skrzydlewska, *et al.* (2002) showed protective effect of green tea against lipid peroxidation in the rat serum and brain.

In this respect, Lee *et al.* (2003) found green tea polyphenol - epigallocatechin gallate was the most potent antioxidant in inhibiting H_2O_2 or ferrous ion-induced lipid peroxidation in the gerbil brain homogenates. Moreover, Yamamoto *et al.* (2006) found intake of green tea catechins for 4 weeks elevated vitamin E and reduced LPO levels in the mucosa of rat large intestine. They also postulated that the metal-binding capability of GTE also extend to the chelation of Pb.

Nitric oxide is a lipophilic and chemically unstable molecule. It is a gaseous substance produced by the nitric oxide synthase (NOS) from L-arginine. Research studies confirmed the distribution of NOS in different brain regions (Vincent, 1995). NO may possess both neurodestructive and neuroprotective properties (Dawson, 1995). NO reacts with superoxide and other ROS to produce peroxynitrite, a highly cytotoxic reactive nitrogen species. Peroxynitrite in turn reacts with and damage proteins, lipids and DNA (Halliwell, 1994). In the present study, the levels of NO were significantly lower in plasma and brain tissue homogenates in Pb treated group than control.

The increased oxidative stress produced as a result of Pb toxicity was well marked by the enhanced LPO production in the plasma and brain tissue (Daniel *et al.*, 2004). Usually the deleterious effects of oxidative stress are counteracted by the natural defense mechanisms that involve enzymes and non-enzymatic scavengers of free radicals (Masso *et al.*, 2007).

Reduced GSH levels and SOD activities in tissues are most commonly used to evaluate Pb induced oxidative damage. The

endogenous GSH, synthesized mainly in the liver, plays an important role in the system of cell defense. GSH is directly associated with the presence of reduced SH groups. It is involved in detoxication of many xenobiotics through conjugation of toxic metabolites (Koegh *et al.*, 1994). In the current study, the levels of GSH were significantly reduced in plasma and brain tissues in Pb treated groups than controls. The decreased GSH levels in tissues after exposure to Pb might result from high affinity of this metal into SH groups. Binding of Pb into SH groups of GSH resulted in a decrease in the GSH oxidative potential (Gurer *et al.*, 1999).

It has been revealed that Pb may affect the antioxidant barrier via inhibiting the activities of enzymes involved in GSH metabolism, such as GST and SOD by blocking their SH groups (Sivaprasad *et al.*, 2004; Patrick, 2006).

In this study, GST activity in brain tissue was significantly decreased in Pb-group compared with control. Meanwhile, GST activity was elevated after administration of GTE to reach control level. In contrary, Bokara *et al.* (2009) reported that GST activity increased with Pb exposure time in brain tissues, showing protection against Pb acetate toxicity.

It is known that SOD requires copper and zinc for its activity and it is believed to play a major role in the first line of antioxidant defense in cell**.** In this study, SOD activities were significantly decreased brain tissue in Pb-treated group compared with control. Meanwhile, a SOD plasma level was decreased but did not reach significant level in Pb-treated group than control. Literatures on the influence of Pb on SOD activity are divergent.

In conclusion, alterations in several indicators of oxidative stress in this animal model of lead intoxication suggested that cellular damage mediated by free radicals may be involved in the pathology associated to lead neurotoxicity. The supplementation with GTE, an antioxidant and chelator, could recover these oxidative damages partly. It is suggested that GTE is a potential complementary agent in the treatment of lead intoxication. But 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 metals, will lead to a clearer and better understanding of the potential health effects of green tea.

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