THE ROLE OF PENICILLAMINE AND GREEN TEA ON BRAIN OF ADULT MALE ALBINO RATS AFTER SHORT TERM CHRONIC LEAD EXPOSURE

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ABSTRACT

Background: Lead is a highly toxic substance, which can produce a wide range of adverse health effects in adults and children can **.**The propensity for lead to catalyze oxidative reactions and generate reactive oxygen species has been demonstrated in multiple studies. **Aim:** The aim of this work was to study the toxic effect of short term chronic lead exposure on lead concentration in blood and oxidative markers in blood. Study the toxic effect of short term chronic lead exposure on brain of adult male albino rats by light and immunohistochmistry. Investigate effect of penicillamine and green tea on these toxicological aspects.**Material and Methods**: eighty adult male albino rats were used in this study. They were equally divided into eight equal groups, each of ten rats were used. The first group was served as control, the second group was given saline , third group were given penicillamine only, fourth group was given green tea only, and fifth groups were given lead acetate .Six, seven and eight groups were given lead acetate and penicillamine , lead acetate and green tea, lead acetate ,penicillamine and green tea respectively, for one month . Rats of all groups were sacrificed and used for the following parameters: Lead level in blood and oxidative stress parameters in blood. Also histopathological examination of brain tissues by light microscope was done. **Results:** The results revealed that rats exposed to lead acetate showed significant increasein Glutathione peroxidase and increase in lead levels in blood. The results revealed also pathological changes in the brain tissues .Supplementation with penicillamine alone or green tea alone induced partial reversal of all tested parameters,while in combined adminstration the result was reached nearly the control values .**In conclusion:** The present study revealed that chronic lead exposure in adult rats induced deleterious effects on brain which reversed partially by supplementation with penicillamine or green tea administration and combined adminstration showed more recovery . **It is recommended** using antioxidants during treatment of chronic lead poisoning. Also, further studies are needed to evaluate the effective dose and duration of antixidant and chelator combination needed for reversal of lead effects.

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INTRODUCTION

Lead is a persistent and common environmental contaminant **(Ferrer, 2003)**.

Lead damages cellular material and alters cellular genetics. The mechanism all of these toxic metals have in common involves oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant. 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 lowers the level of available sulfhydryl antioxidant reserves in the body **(Ercal et al.,2001).**

Lead is toxic to many organs and tissues including the hematopoitic system, nervous system ,heart, bones, intestines, kidneys and reproductive system. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behavior disorders. Symptoms include abdominal pain, headache, irritability, and in severe cases seizures, coma, and death **(Guidotti and Ragain, 2007).**

Green tea extract (GTE) due to its content of catechins reveals strong antioxidative activity, which is manifested by its ability to inhibit free radical generation, scavenge free radicals and chelate transition metal ions that catalyze free radical reactions **(Ostrowska and Skrzydlewska, 2006)**.

D-Penicillamine is chelating agent. It is a metabolite of penicillin, although it has no antibiotic properties. It is prescribed for the

treatment of Wilson's disease and cystinuria and can be prescribed to bind with and remove heavy metals from the blood such as lead, copper, mercury, arsenic, and gold **(Colalipour et al., 2007).**

AIM OF THE WORK

The aim of this work was to:

1-Study the toxic effect of short term chronic lead exposure on lead concentration in blood and oxidative markers in blood.

2-Study the toxic effect of short term chronic lead exposure on brain of adult male albino rats by light, and immunohistochmistry.

3-Investigate effect of administration penicillamine alone, green tea alone and their combination on these toxicological aspects.

MATERIAL& METHODS

Material

[A] Chemicals

1)Lead acetate:

Lead acetate ($C_4H_6O_4Pb_3H_2O$) in the form of white crystals manufactured by El Nasr Pharmaceutical Chemical Company. Importer's name: El-Goumhouria Co., Egypt.

2)D-Penicillamine:

In the form of white powder for laboratory use only, 98-101%, (CH3)2C(SH)CH(NH2)CO2H Manufactured by Sigma aldric chemic, st louis, Germany. Importer's name: Sigma life science Co., Egypt.

2) Green tea.

[B] Animals

This study was carried out on 80 adult male albino rats, their weights ranged from 150 -200 gms, they were obtained from animal's house, Faculty of Veterinary Medicine, Zagazig University. Before commencing the experimentation, all animals were subjected to 14 days period of passive preliminaries in order to adapt themselves to their new environment, to ascertain their physical well being and to exclude any diseased animals.

Animals were divided into 8 groups each of 10 rats as follow:

Group I (negative control group): Rats of this group were given no medications to measure the basic tested parameters.

Group II (positive control group): Rats were given 1ml of saline once daily by gavage for one month, 24hours after the last dose rats of this group will be sacrified & used for measuring the testing parameters.

Group III (D-D-penicillamine treated group): Rats were given penicillamine at therapeutic dose 25mg/kg of D- penicillamine dissolved in 1 ml saline on empty stomach once daily orally by gavage for 1 month **(Golalipour et al., 2007).**

Group IV (Green tea): Rats were givengreen tea in drinking water at a concentration of (1.5% w/v) for one month **(Abdel-Moneim et al., 2014).**

Group V (lead treated group): Rats were given Lead acetate was given orally at a dose of 100 mg/kg b. wt for one month**.**

Group VI (lead & D-penicillamine treated group): Rats were given lead acetate for 1 months , 24hours after the last dose rats of this group was given penicillamine for 1 month in the same previous doses.

Group VII (lead & green tea): Rats were given lead acetate for one month followed by green tea for one month. **Group VIII (lead & D-penicillamine and green tea treated group):** Rats were given lead acetate for 1 months, 24hours after the last dose than rats was given penicillamine, and green tea was given for 1 month in the same previous doses.

Rats were weighed every week and the doses were adjusted according to the changes in the body weight.

Methods:

At the end of the experimental periods, rats of all groups were used to measure the following parameters.

[A] Biochemical parameters:

Venous blood samples were obtained from animals by means of capillary glass tubes from the retro-orbital plexus under light ether anesthesia (**Semler, 1992)**.

The collected blood was used as following:

1- Heparinized blood samples were centrifuged for 10 minutes at 3000 r.p.m. Then the plasma was aspirated and RBCs were washed four times with 3ml of 0.9% NaCl and centrifuged. Samples were maintained at (-20°C) to be used for estimation of reduced glutathione **(Paglia and Valentine ,1967)** and glutathione peroxidase (GSHpx) enzyme **(Pleban et al. 1982)**

2- Blood lead level (BLL):

Sample of whole blood samples were digested with an acid mixture consisting of ultra pure HNO_3: HCl: H_2SO_4 (6:1:1). The resulting carbon free residue was dissolved in 10ml, 1 M of HNO_3 (by dissolving 31.2 ml of HNO_3 in 500ml distilled water) and transferred to poly-ethylene tubes for estimation of blood lead level by atomic absorption spectrophotometer **(Burtis and Ashwood, 2001).**

[B] Tissue parameters:

Brain were immediately dissected out and grossly inspected to assess any gross abnormalities then washed with cold normal saline and used for histopathological study.

(1) Light microscope examination:

The brain were fixed in 10% formalin saline. After fixation, tissues were embedded in paraffin blocks and processed for the preparation of 5 u. thickness sections. These sections were subjected for Hematoxyin and Eosin stains **(Horobin and Bancroft, 1998)** and then examined by light microscope.

(2) immuno-histochemical study:

Immediately after dissection, immunohistochemical reactions were carried out on sections of brain and heart of adult male albino rats using caspase 3**according to Krajewska et al. (1997).**

Statistical analysis:

Data were analyzed by Statistical Package of Social Science (SPSS), software version 22.0 **(SPSS Inc., 2013).**.

RESULTS

As regard the control groups (negative control group and distilled water group): There was a non significant difference between them as regard lead level in blood and oxidative stress parameters in blood**(Tables 1).**

Also there was a non significant difference between the control groups, penicillamine and green treated groups as regard lead level in blood and oxidative stress parameters in blood **(Tables 2).**

So the negative control group was chosen to compare with the treated groups (lead group, lead & D-penicillamine ,lead & green tea group group. and, lead & D-penicillamine with green tea group).

I)-Hematological parameters results:

(2)Lead level in blood:

There was a highly statistically significant increase in the mean values of in lead group when compared with the negative control group. When the mean values of BLL in rats of lead & penicillamine group, lead &green tea group and, lead & penicillamine with green tea group are compared to those recorded in rats of lead group there was a highly statistically significant decrease in BLL **(Table 3)**.

(3) Oxidative stress markers:

Mean values of reduced glutathione and glutathione peroxidase show highly significant difference in lead treated group when compared with negative group. Reduced glutathione show highly significant increase in lead and penicillamine, lead and green tea and lead and penicillamine with green tea treated group when compared with lead treated group**.**Glutathione peroxidase show highly significant decrease in lead and penicillamine, lead and green tea and lead and penicillamine with green tea treated group when compared with lead treated group **(Table 4) .**

III)-Histopathological changes:

1-Light microscopic examination:

Specimens from brain of lead treated rats revealed histopathological changes in the brain by light microscope which revealed many distorted cells with deeply stained shrunked nuclei and cytoplasm surrounded by vacuolated pale areas. Few pyramidal cells appeared normally **(fig2)**.

The pathological changes were partially reversed by treatment of lead with either D-penicillamine **(fig.3)** or gree tea **(fig.4)**. While, combined treatment of lead with both D-penicillamine and green tea revealed nearly normal structures of the examined organs**(Fig.5).**

2-Immunohistochmistry:

Negative immune reaction for caspase-3 was detected in control groups **(fig.6)** .While lead treated group showed positive immune reaction for caspase-3 in most of nerve cells **(fig.7)**. positive immune reaction for caspase-3 in some nerve cells in group VI (lead & D-penicillamine group)**(fig.8)** and group VII (lead & green group) were noticed (**fig.9)** ,while Group VIII (lead & D-penicillamine with green tea treated group) showed negative immune reaction**(fig.10)**.

Table (1): Statistical comparison between the negative control group (I) and distilled water group group (II) as regard the lead level in blood and oxidative stress parameters in blood by t- test.

Group / Parameter	Negative control group(I)	Distilled water group(II)	F	P
	Mean ±SD	Mean ±SD		
BLL (µg/dl)	5.24±0.08	5.21±0.09	**0.621**	>0.05 NS
Gpx (u/gm Hb)	29.73±2.49	28.91±2.5	**0.540**	>0.05 NS
Reduced glutathione (u/gm Hb)	0.202±0.06	0.201±0.05	0.002	>0.05 NS

Table (2): Statistical comparison between the negative control group (I), D-penicillamine group (III) and green tea group (IV) as regard the lead level in blood and oxidative stress parameters in blood by ANOVA test.

Group / Parameter	Negative control group (I)	DPenicillamine Group (III)	Green tea Group (IV)	F	P
	Mean ±SD	Mean ±SD	Mean ±SD		
BLL (µg/dl)	5.24±0.08	5.23±0.08	5.24±0.09	**0.048**	>0.05 NS
Gpx (u/gm Hb)	29.73±2.49	28.87±2.39	29.02±2.41	**0.357**	>0.05 NS
Reduced glutathione (u/gm Hb)	0.202±0.06	0.201±0.05	0.203±0.06	0.002	>0.05 NS

Table (7): Comparison between negative control group, lead group, lead &penicillamine group, lead &green tea group and, lead &penicillamine with green tea group as regard BLL by ANOVA test and LSD e.

Group / Parameter	Negative control group	Lead group	Lead & penicillamine	Lead & green tea group	Lead & penicillamine with green tea group	F	P
	Mean±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
BLL (µg/dl)	5.24±0.08	111.3±11.14* A	18.51±7.4* Ab	20.14±8.6* abc	12±8.5* Ac	**296.734**	<0.001***

*** = Highly Significant (p<0.001).
* Significant compared with negative control group.
a = Significant compared with lead group.
b = Significant compared with lead & D- penicillamine with green tea group.
c = Significant compared with lead & D- penicillamine group.

Table (8): Comparison between negative control group, lead group, lead &penicillamine group, lead &green tea group and, lead & penicillamine with green tea group as regard oxidative marker by ANOVA test and LSD .

Group / **Parameter**	**Negative control group**	**Lead group**	**Lead & penicillamine group**	**Lead & green tea group**	**Lead & penicillamine with green tea group**	**F**	**P**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**		
Reduced glutathione (u/gm Hb)	0.202±0.06	0.102±1.73*	0.151±1* Ab	0.156±1* ab	0.198±1* Ac	**0.014**	<0.001 ***
Glutathione peroxidase (u/gm Hb)	29.73±2.49	56.01±5.36*	42.36±1.62* Ab	35.55±1.05* abc	30.50±2.72* Ac	**127.720**	<0.001 ***

*** = Highly Significant (p<0.001).
* Significant compared with negative control group.
a = Significant compared with lead group.
b = Significant compared with lead & D- penicillamine with green tea group.
c = Significant compared with lead & D- penicillamine group.

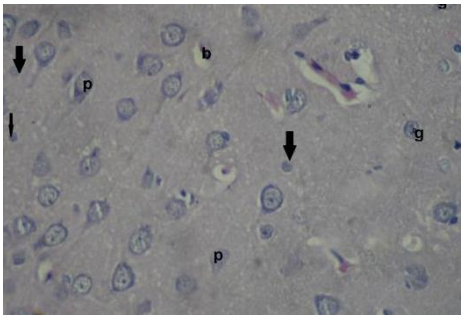

Fig. (1): A photomicrograph of a section in the internal granular and internal pyramidal layers of frontal cortex of a control rat showing few granular cells (g) and pyramidal cells (p) with vesicular nuclei, basophilic cytoplasm and processes. The surrounding neuropil contains nerve fibers, glail cell (arrow) and blood vessels (b) (Hx & E X 400).

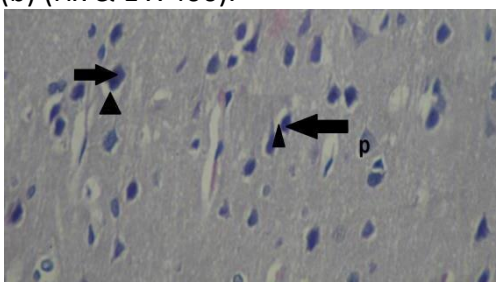

Fig. (2): A photomicrograph of a section from frontal cortex of a lead treated rat showing many distorted cells with deeply stained shrunken nuclei and cytoplasm (arrow).Unstained vacuolated areas are surrounding cells (arrow head). Few pyramidal cells appear normally (p) (Hx & E X 400).

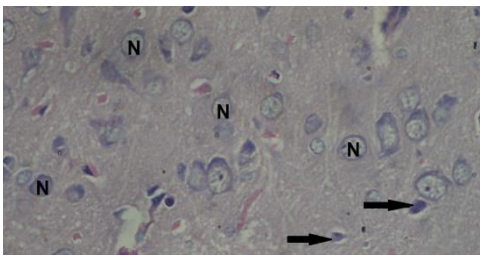

Fig. (3): A photomicrograph of a section from frontal cortex of a lead and D-penicillamine treated showing that some nerve cells appear normally with large vesicular nuclei (N) .Other cells appear distorted with deeply stained shrunked nuclei and cytoplasm (arrow) (Hx & E X 400).

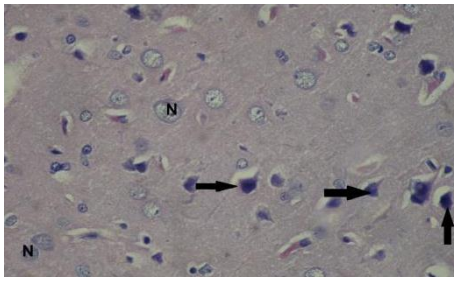

Fig. (4): A photomicrograph of a section from cortex of the cerebrum of a lead and green tea treated rat showing that most nerve cells in the cortical layer appear normally with large vesicular nuclei (N) .Some cells appear distorted with deeply stained shrunked nuclei and cytoplasm (arrow) (Hx & E X 400).

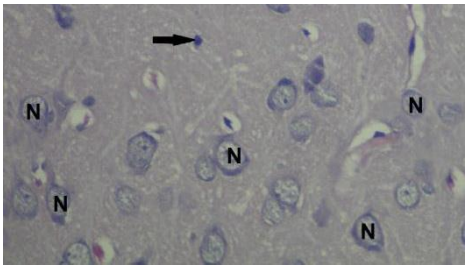

Fig. (5): A photomicrograph of a section from the cortex of the cerebrum of a lead and combined D-penicillamine with green tea treatment rat showing that most nerve cells in the cortical layer appear normally with large vesicular nuclei (N) .Few cells appear distorted cells with deeply stained shrunked nuclei and cytoplasm (arrow). (Hx & E X 400

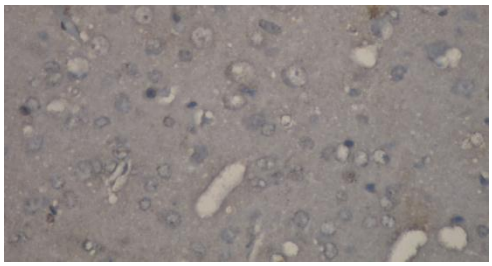

Fig. (6): A photomicrograph of a section in the internal granular and internal prramidal layers of frontal cortex of a control rat showing no immune reaction for caspase-3,X400

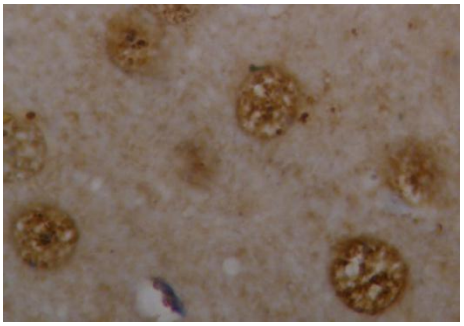

Fig. (7): A photomicrograph of a section in the internal granular and internal prramidal layers of frontal cortex of a lead treated group showing positive immune reaction in the most of nerve cell for caspase-3,X400

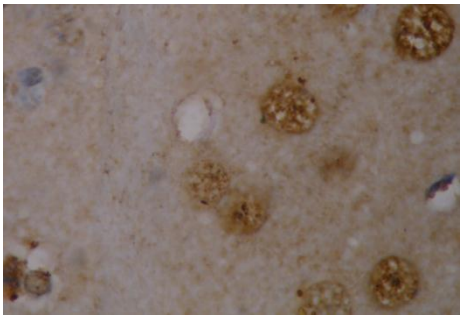

Fig. (8): A photomicrograph of a section in the internal granular and internal prramidal layers of frontal cortex of a lead & D-pencillamine treated group showing positive immune reaction in few nerve cell for caspase-3,X400

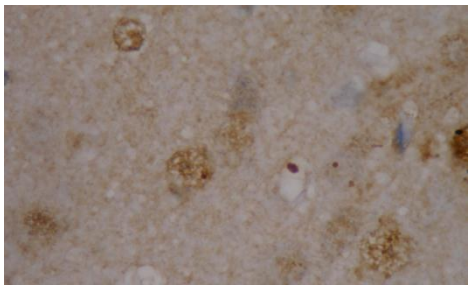

Fig. (9): A photomicrograph of a section in the internal granular and internal prramidal layers of frontal cortex of a lead & green tea treated group showing positive immune reaction in few nerve cell for caspase-3,X400

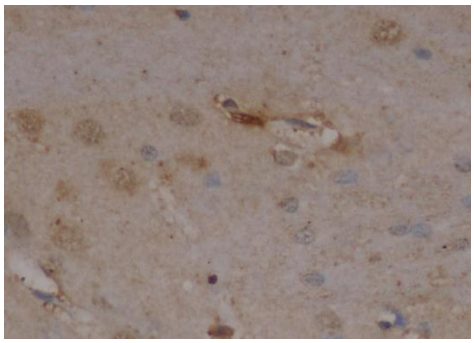

Fig. (10): A photomicrograph of a section in the internal granular and internal prramidal layers of frontal cortex of of a lead and combined D-penicillamine with green tea group showing positive immune reaction in few nerve cell for caspase-3,X400

DISSCUSION

For centuries, lead toxicity has been one of the most significant preventable causes of neurologic morbidity from an environmental toxin. It interferes with a number of body functions primarily affecting the central nervous, hematopoietic, hepatic and renal system producing serious disorders. Acute toxicity is related to occupational exposure and is quite uncommon. Chronic toxicity on the other hand is much more common **(Murata et al., 2009).**

The results of the present study revealed statistically significant increase in the mean values of BLL and lead level in urine in lead group when compared with the control group**.**

The main tool in diagnosing and assessing the severity of lead poisoning is laboratory analysis of (BLL).Also lead level in urine is considered as non invasive manover to detect lead toxicity **(Mycyk et al. 2005)**.

Although no blood level of lead is considered safe, the Centers for Disease Control and Prevention (CDC) has established 10 µg/dL as the level of concern. Approximately 9% of children aged 1-5 years have blood levels higher than 10 µg/dL. Because low socioeconomic status is also a risk factor for lead exposure, children in inner cities are at highest risk. In some rural areas of the United States, 20% of children have been reported to have levels higher than 10 µg/dL **(Norman et al., 1994).**

The results of the present study revealed that mean values of reduced glutathione and glutathione peroxidase show highly significant difference in lead treated group when compared with negative group**.**

The major mechanism of lead toxicity is due to increased generation of reactive oxygen species (ROS) and interference with generation of antioxidants. Lead causes the generation of ROS like hydroperoxide, hydrogen peroxide, and singlet oxygen. ROS are stabilized by glutathione in the body. Ninety percent of glutathione in the cell exists in reduced form and 10% in oxidative form, and it typically acts as an antioxidant defense mechanism. Glutathione stabilizes ROS, and after being converted (oxidizing) to glutathione disulfide, it is reduced back to GSH by glutathione reductase. Lead inactivates glutathione by binding to GSH's sulfhydryl group, which causes GSH replenishment to become inefficient, thereby increasing oxidative stress **(Flora et al., 2012).**

Lead has also been shown to both elevate and suppress blood levels of the antioxidant enzymes as glutathione peroxidase (GHPx). Elevations of these enzymes have been seen in lower levels of exposure, while suppression can occur at higher exposure levels over longer periods of time **(Han et. al., 2005)**

According to **Patrick (2006)** lead-exposed workers with high blood lead levels (over 40 µg/dL) had significant reductions in blood GPx as compensatory reaction for increased lipid peroxidation.

According to **Hunaiti et al. (1995)** concentrations of glutathione in the blood was significantly lower than control levels both in animal studies of lead exposure and in lead-exposed children and adults.

The results of the study revealed that supplementation of rats exposed to lead with D-penicillamine or green tea extract individually or in combination resulted in reversal of all tested parameters and pathological changes.

These findings are supported by the results of other investigators, who found that According to **American Academy of Pediatrics (1995)** D-penicillamine may be used to treat heavy metal poisoning caused by lead alone for minor intoxication or as adjunctive therapy after calcium EDTA or BAL.

According to **Beattie, (1977).**D-penicillamine sulfhydryl group combines with lead to form ring compounds increasing its elimination.

Many investigators proposed that one possible mechanism of Pb toxicity is the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species **(Wang et al. 2007).**

Adonaylo and Oteiz, (1999) reported that Pb-associated tissue injury in the vital organs is resulted from the oxidative stress.

Some studies suggested potential role of antioxidants to ameliorate Pb toxicity **(*Gurer* et al., 1998).**

Green tea extract (GTE) due to its content of catechins reveals strong antioxidative activity, which is manifested by its ability to inhibit free radical generation, scavenge free radicals and chelate transition metal ions that catalyze free radical reactions **(Ostrowska and Skrzydlewska, 2006).**

Meki et al. (2011) concluded that the treatment of rats with GTE combined with Pb could enhance antioxidant/ detoxification system which consequently reduced the oxidative stress. The beneficial effect of GTE in the improving of antioxidant status was associated with reduction of Pb burden in the tissue organs, thus potentially reducing Pb toxicity and tissue damage.

The results of the study revealed that Macroscopic examination of the brain of all the studied groups revealed normal appearance with no changes in size or abnormal masses compared with the control groups. Cut sections were apparently normal**.** Microscopic examination of the brain specimens of rats of lead treated (V) group revealed the following histopathological changes: distorted cells with deeply stained shrunked nuclei and cytoplasm.

These results were in consistent with **Kang et al. (2004)** who stated that microscopical examination of the brains of low- and medium-dose lead treated groups showed no definite histopathological abnormalities. However, the brains of animals in the high-dose lead treated group showed diffuse vacuolar degeneration and increased neovascularization of the cerebral white matter and hippocampus as well as cerebellar Purkinje cell degeneration. In addition, neuronal degeneration and mild spongy changes in brain stem nuclei.

According to **Sharma (2005),** microscopic lesions in lead toxicity were extravascular red blood cells, and an enlarged extracellular space**.**

According to **Eltony et al. (2010)**, brain showed shrunken and irregular outline Purkinje cells and some Purkinje cells were fallen off leaving empty spaces .

On the present study, microscopic examination of the brain specimens of the rats of lead & D-penicillamine group (VI) and lead & Green tea extract (VII) showed that some nerve cells in the cortical layer appear normally with large vesicular nuclei with some distorted cells with deeply stained shrunked nuclei and cytoplasm**.** While lead & penicillamine with green tea treated group (VIII) showed that most nerve cells in the cortical layer appear normally with large vesicular nuclei with few distorted cells with deeply stained shrunked nuclei and cytoplasm**.**

Lead-induced oxidative stress had been identified as the primary contributory agent in the pathogenesis of lead poisoning. Oxidative stress had also been implicated in specific organs with lead-associated injury, including liver, kidneys, and brain tissue. ROS generated as a result of lead exposure had been identified in lung, endothelial tissue, testes, sperm, liver, and brain **(Hsu and Guo, 2002).**

Chelating agents have antioxidant properties as D-penicillamine has been shown to act as a free radical scavenger **(Ercal et al., 1996).**

These results were in consistent with **Meki et al. (2011)** who stated that the antioxidant status was improved in almost the studied tissues in lead with Green tea extract treated rats comparing to lead treated rats.

Lead acetate administration induced loss of body weight and decreased concentration of reduced glutathione and SOD activity in brain tissues as well as significantly high DNA fragmentation and pathological changes. Co-administration of green tea with lead acetate significantly alleviated these adverse effects **(Khalaf et al., 2012).**

Multiple studies had assessed the effect of antioxidants used in conjunction with the chelators used treatment of lead toxicity **(Sivaprasad et al., 2004).**

Oriyanhan et al. (2005) reported a synergistic effect between antioxidants and pharmaceutical chelating agents**.**

In the present study, lead treated group (VI) showed showed positive immune reaction in most of nerve cells when compared with control group. On the other hand, positive immune reaction in some of nerve cells in group VI (lead &penicillamine group) and group VII (lead & green tea group) ,while Group VIII (lead & penicillamine with

green tea treated group) showed negative immune reaction.

Caspase-3 is activated during neuronal programmed cell death **(Armstrong et al., 1996).**

These results were in consistent with **Dribben et al. (2011)** who stated that the ability of lead exposure to produce apoptosis in the neonatal mouse brain was assessed histologically 8-24h after treatment using activated caspase-3 immunohistochemistry.

Reversal of lead-induced neuronal apoptosis by D-penicillame , green tea and combined treatment were noticed through reduction of positive reaction in many nerve cells . These results were in consistent with **Flora et al. (2007)** who indicated that lead caused a significant increase in reactive oxygen species, neuronal nitric-oxide synthetase, and intracellular free calcium levels along with altered behavioral abnormalities in locomotor activity, exploratory behavior, learning, and memory that were supported by changes in neurotransmitter levels. A fall in membrane potential, and release of cytochrome C indicated mitochondrial-dependent apoptosis. Most of these alterations reverted toward normal level following chelation ,antioxidant and combined treatment.

In conclusion, in the present experiment chronic lead exposure in adult rats induced deleterious effects on hematopoietic system, lipid profile, BLL and lead level in urine, oxidative marker , brain of adult albino rats. These effects may be due to lipid peroxidation and decreased activity of antioxidant enzymes. Moreover, these effects were reversed partially by supplementation with D-penicillamine and green tea, and reversed nearly to control values with their combined administration.

It is recommended, to use antioxidants during treatment of chronic lead poisoning. Also, further studies are needed to evaluate the effectiveness of D-penicillamine and green tea and determination of exact dose and time needed for reversal of lead effects.

REFERENCES

1. **Abdel-Moneim, A.H.; Meki, A. ; Gabr, A. M. A. S.; Mobasher, A. A. and Lutfi, M. F. (2014):** The Protective Effect of Green Tea Extract against Lead Toxicity in Rats Kidneys. Asian Journal of Biomedical and Pharmaceutical Sciences; 4(39): 30-34.
2. **Ademuyiwa**,O.; **Ugbaja, R. N.**, **Idumebor, F.** and **Adebawo, O. (2005):** Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta, Nigeria**.** Lipids in Health and Disease, **4**:19**.**
3. **Adonaylo, V.N and Oteiza, I. P. (1999):** Lead intoxication defenses and oxidative damage in rat brain. Toxicology, 135: 77-85.
4. **American Academy of Pediatrics, Committee on Drugs (1995):** Treatment guidelines for lead exposure in children. Pediatrics, 96:155-60.
5. **Armstrong, R.C.; Aja, T. and Xiang, J. (1996):** Induced activation of the cell death-related protease CPP32 is inhibited by Bcl-2 and by ICE family protease inhibitors. J .Biol. Chem., 271:16850:5
6. **Beattie, A.D. (1977):** The use of D-penicillamine for lead poisoning. Proc Roy Soc Med. , 70: 43-5.
7. **Burtis, C.A. and Ashwood, E.R. (2001):** Tietz fundamentals of clinical chemistry, 5th ed., W.B. Saunders, USA, pp. 75.
8. **Dribben, W.H.; Creeley, C.E. and Farber, N. (2011):** Low-level lead exposure triggers neuronal apoptosis in the developing mouse brain. Neurotoxicol. Teratol., 33(4):473-80.
9. **Eltony, S. A.; anal Othman, M. A.; and Mohamed, A. A. (2010):**Histological Study on the Effect of Low Level Perinatal Lead Exposure on the Cerebellar Cortex of Adult Male Albino Rat. Egypt. J. Histol. 33(4): 781 – 97.
10. **Ercal, N.; Treeratphan ,P.and Hammond ,T.C.(1996):** Invivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-

acetylcysteine. Free Radic. Biol .Med.;21:157-61.

11. **Ferrer, A. (2003):** Metal poisoning .An sist saint navar., 26:141-53.
12. **Flora, G.; Gupta, D.and Tiwari, A. (2012):** Toxicity of lead: A review with recent updates. *Interdiscip. Toxicol.* , 5 (2):47-58.
13. **Flora, S.J.; Saxena, G. and Mehta, A. (2007):** Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: role of reactive oxygen species and intracellular Ca.J .Pharmacol. Exp. Ther., 322(1):108-16.
14. **Golaipour, M.J.; Roshandel, D.; Ghafari, S.; Kalavi, M .and Kalavi, S. (2007):** Effect of lead intoxication and d penicillamine on hematological indices in rats. INT .J.MORPHOL., 25(4):717-22.
15. **Guidotti, T.L.and Ragain, L. (2007):** Protecting children from toxic exposure: three strategies. Pediatric clinics of North America, 54 (2): 227–35.
16. **Gurer, H.; Ozgunes, H.and Neal, R. (1998):** Antioxidant effects of N-acetylcysteine and succimer in red blood cells from lead-exposed rats. Toxicology ,128:181-89.
17. **Han, S.G.;Kim, Y.and Kashon, M.L.(2005):** Correlates of oxidative stress and free-radical activity in serum from asymptomatic shipyard welders. Am J Respir Crit Care Med., 172:1541-8.
18. **Horobin, R.W. and Bancroft, J.D. (1998):** Hematoxylin and eosin as an oversight stain. In: Trubleshooting Histology stains, 1st ed., Churchill Livingstone Press, San Francisco, pp. 88- 93.
19. **Hsu, P.C. aand Guo, Y.L.(2002):** Antioxidant nutrients and lead toxicity. Toxicology ,180:33-44.
20. **Hunaiti ,A.;Soud, M.and Khalil, A.(1995):** Lead concentration and the level of glutathione, glutathione S-transferase, reductase and peroxidase in the blood of some occupational workers from Irbid City ،Jordan. Sci Total Environ., 170:95-100.
21. **Khalaf, A.A. ;Moselhy, W.A.and Abdel-Hamed, M.I.(2012):** The protective effect of green tea extract on lead induced oxidative and DNA damage on rat brain. Eurotoxicology,33(3):280-9.
22. **Kang, J.K.; Sul, D.; Kang,J,K.; Nam,S.; Kim, H.and Lee,E.(2004):** Effects of Lead Exposure on the Expression of Phospholipid Hydroperoxidase Glutathione Peroxidase mRNA in the Rat Brain. Toxico Scien., 82(1):228-36.
23. **Krajewska, M.; Wang, H.G.; Krajewski, S.; Shabaik, A.; Gascoyne, R. and Reed, J. C. (1997):** Immunohistochemical analysis of in vivo pattern of expression of CPP32 (Caspase 3), a cell death protease. Cancer Res., 57: 1605-1631.
24. **Meki, A.R. Alghasham, A.and EL-Deeb,E.S.(2011):** Effect of green tea extract on lead toxicity in different organs of rats .Int. J. Health Sci. , 5(2 Suppl 1): 12–15.
25. **Murata ,K.; Iwata, T.; Dakeishi, M.and Karita, K.(2009):** Lead toxicity: does the critical level of lead resulting in adverse effects differ between adults and children?. *J. Occup. Health*., 51(1):1-12.
26. **Mycyk, M.; Hryhorczuk, D.and Amitai, Y. (2005** Lead. In :Pediatric Toxicology: Diagnosis and Management of the Poisoned Child by Erickson, TB; Ahrens, WR; Aks, S; Ling, L.. McGraw-Hill Professional.p. 464.
27. **Norman, E.H.; Bordley, W.C.; Hertz-Picciotto, I.and Newton, D.A. (1994):** Rural-urban blood lead differences in North Carolina children. *Pediatrics*. , 94(1):59-64.
28. **Oriyanhan, W.; Yamazaki, K.and Miwa ,S.(2005):** Taurine prevents myocardial ischemia/reperfusion-induced oxidative stress and apoptosis in prolonged hypothermic rat heart preservation. Heart Vessels ,20:278-85.
29. **Ostrowska, Jand Skrzydlewska, E.(2006):** The comparison of effect of catechins and green tea extract on

oxidative modification of LDL in vitro. Adv. Med. Sci., 51:298-303.

30. **Paglia, D.E. and Valentine W.N. (1967):** Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase . J. Lab. Clin. Med., 70: 58- 169.
31. **Patrick, L. (2006):** Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. Alternative medicine review : a journal of clinical therapeutic, 11 (1): 2–22.
32. **Pleban,P.A.; Munyani, A.; and Beachum, J. (1982) :** Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. Clin. Chem., 28:311-6.
33. **Semler, D.E. (1992):** The rat toxicology. In: animal models in toxicology, by Gad, S.C. and Chengelis, C.P., Marcl Dekker Inc. New York, pp 21- 75.
34. **Sharma, R.K. and Agrawal, M. (2005):** Biological effects of heavy metals: an overview. J. Environ. Biol., 26: 301- 13.
35. **Sivaprasad ,R.; Nagaraj ,M.and Varalakshmi, P.(2004)** :Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J. Nutr .Biochem. ,15:18-23.
36. **SPSS Inc (2013): SPSS for windows, version 22.0. Chicago, SPSS Inc.** http://www.unimunester.de/imperia/md/content/ziv/service/softwar e/spss/handbuecher/englisch/spss_brief_guide_22.0.pdf**.**
37. **Wang, C. J.; Liang, C. ;Zhang, Y.; Bi, X.; Shi and Shi, Q. (2007):** Effect of ascorbic Acid and thiamine supplementation at different concentrations on lead toxicity in liver. Ann. Occup. Hyg., 51(6): 563-9.
38. **Wang, Q.; Luo, W.; Zheng, W.; Liu ,Y.;Xu ,H.;Zheng ,G. Dai, Z.; Zhang ,W.; Chen ,Y.and Chen ,J.(2007):** Iron supplement prevents lead-induced disruption of the blood-brain barrier during rat development. Toxicol Appl Pharmacol., 15 (1):33-41.