EFFECT OF CATECHIN AND CALCIUM DISODIUM ETHYLENE DIAMINE TETRA ACETIC ACID (CANA2EDTA) ON LEAD TOXICITY IN RATS: RESIDUAL AND HISTOPATHOLOGICAL STUDIES

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ABSTRACT

Heavy metals induce toxic effects on different systems. Among heavy metals, lead represents the main environmental poison, is a non essential toxic heavy metal widely distributed in the environment and causes neurological impairment. The present study was conducted to evaluate the efficacy of both Catechin and CaNa2EDTA in treatment of long-term lead. Eighty male albino rats weighting 100-150 g (10-12 weeks old) were randomly divided into 4 groups (20 each). Group 1 was left without any treatment and served as control group. The other groups (G2, G3 and G4) were exposed to lead acetate in drinking water at a concentration of 30 mg/l for 3 months. G2 was used as positive control group. G3 was divided into three subgroups (a, b and c) and treated with catechin in drinking water 49 mg/l for 7 days after 1st, 2nd and 3rd month post Pb exposure. G4 was divided into three subgroups (d, e & f) and treated through IP injection with CaNa2EDTA in a dose of 50 mg/kg body weight, for 5 days after 1st, 2nd and 3rd month post Pb exposure lead exposure. Six rats were taken randomly after 30, 60 and 90 days from negative and positive controls, 37, 67, 97 days from rats treated with catechin and 35, 65 and 95 days from rats treated with CaNa2EDTA. Rats were anesthetized with ether and scarified for blood and brains collection for metal residue and histo-pathological examination. The results revealed that catechin and CaNa2EDTA had ameliorated the toxic effects of lead through minimizing the residues of metals and restoration of the histopathological changes in the brain tissues.

Key Words: *Metals-histopathological examination, catechin, CaNa2EDTA- lead-rats.*

INTRODUCTION

The main environmental sources of Lead (Pb) are leaded gasoline, lead shots or bullets, soil, dust, toys, lead acid batteries, cosmetics and paints (Thuppil & Tannir, 2013). Pb is an

environmentally persistent toxin that causes neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological disorders (Patrick, 2006b). Pb interferes with the metabolism of certain essential elements as copper (Cu) and iron (Fe) by affecting their absorption, distribution and bioavailability in the body; and can inhibit DNA protein (Gustafson *et al.*, 1989). Adequate intake of Cu provides protection against Pb, whereas higher intake of Cu increases Pb absorption (Miller *et al.*, 1989). Cu and Fe can reduce the severity of Pb toxicity by decreasing the body level of Pb, altering absorption of Pb or restoring Pb-induced biological alterations (Petering, 1978). High Fe in diet significantly alter the toxic effects of low-level Pb ingestion in rats (Six & Goyer, 1972). The toxic effects and the body uptake of Pb are greatly minimized when dietary Cu, and Fe are adequate and an inverse relationship between dietary Cu and Fe with the absorption of Pb (Klauder & Petering, 1975). Pb interferes with mitochondrial energy metabolism, which is necessary to reduce ferric iron (Fe^{3+}) to ferrous iron ($Fe2+$) before insertion of iron into the porphyrin ring. When iron deficiency is present, ferrochelatase is more sensitive to these effects of Pb and results in depression of hematopoiesis. Therefore, iron supplementation prevents this toxic effect of Pb on hematopoiesis. Transferrin is the major iron-transport protein in serum and other biological fluids capable of transporting various metals. Pb inhibited transferrin endocytosis and iron transport across the cell membrane of reticulocytes (Mudipalli, 2007).

Pb exposure induces edema in the hippocampus and between the cells associated with focal gliosis in the cerebrum, vacuolization in cerebellum, neuronal degeneration and gliosis in medulla oblongata. In consistence, Amal & Mona (2009) found that brain of rats received 100 mg/l Pb acetate in drinking water for 3-6 weeks, showed pyknosis of neurons associated with focal gliosis and focal cerebral hemorrhage. Deveci (2006) observed degeneration in some neurons and dilatation in the lumen of the blood vessels in brain of rats treated with Pb acetate at a dose of 500 ppm via drinking water for 60 days. Also, treatment of rats with Pb at dose level of 50 mg/kg b.w. caused degeneration of neurons in cerebrum (Sidhu & Nehru, 2004).

Tea from the plant Camellia sinensis, is consumed in different parts of the world as green or black tea (Chacko *et al*., 2010). The health-promoting effects of green tea are mainly attributed to its polyphenol content (Khan & Mukhtar, 2007). Most of the green tea polyphenols are flavanols, known as catechins. Green tea, its extract, and its isolated constituents are effective in preventing oxidative stress (Babu *et al*., 2006) and neurological problems (Unno *et al*., 2007). This effect has been attributed to the presence of high amounts of polyphenols, which are potent antioxidants (Chacko *et al*., 2010). The capacity of catechins to act as antioxidants depends upon their molecular structure, their general structure includes a diphenylpropane moiety composed of two or more aromatic rings (A & B), each having at least one aromatic hydroxyl group connected via carbon chain (Larson *et al.*, 2012). The metal chelating ability of catechins depend on both the hydroxyl groups of ring-B and the 5-hydroxy group of ring-A. Catechins are free radical scavengers and can scavenge both hydroxyl and superoxide radicals as well as lipid free radicals and peroxyl radicals and also they have the ability to chelate metals such as Cu^{2+} and Fe^{3+} due to their catechol structure, and form inactive complexes (Sutherl *et al*., 2006).

The ability of tea polyphenols to chelate some metal ions (Fe & Cu) may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation (Rice-Evans *et al.*, 1997). Tea catechins can affect iron absorption, particularly in individuals at risk of Fe deficiency because they have an affinity for Fe (Nelson & Poulter, 2004), and green tea can cause a significant decrease of the Fe bioavailability from the diet (Hamdaoui *et al.*, 2003).

Green tea ingestion over a long period does not affect the apparent absorption of Cu, whereas it decreases that of Zn and increases that of manganese (Zeyuan *et al.*, 1998). Catechin intake does not affect the plasma concentration of these ions (Record *et al.*, 1996) but they have the potential to affect absorption and metabolism of ions because catechins interact with a variety of metal ions (Mira *et al.*, 2002).

CaNa2EDTA is commonly used chelating agent. It is a derivative of ethylenediamine tetraacetic acid; a synthetic polyamino-polycarboxylic acid which was used for the treatment of metal poisoning and had been the mainstay of chelation therapy for many years (Kalia &Flora, 2005).

1- MATERIALS

(a) Chemicals

- Catechin, ($C_{15}H_{14}O$) and 98% purity was obtained from Sigma chemicals Co., USA.
- Calcium disodium ethylenediamine tetraacetic acid (Calcium disodium EDTA), $C_{10}H_{12}N_2O_8Ca$ $Na_2.2H_2O$ and purity 95%, was obtained from LAB-chemical, India.
- Lead acetate [$Pb(CH_3COO)_2.3H_2O$] with molecular weight of 379.33 was obtained from EL Nasr pharmaceutical chemicals Co., Egypt.
- All other chemicals and reagents were of the highest purity commercially available.

(b) Animals

Eighty male albino rats weighting (100-150 g of 10-12 weeks old) were obtained from the laboratory animals house, Faculty of Medicine, Assiut University. The rats were housed in plastic cages, five rats each. Animals were acclimatized to laboratory condition two weeks before the experiment and fed commercial pellet rat feed. Feed and water were available add libitum, suitable temperature and lighting cycle of 12 hours (light/dark) were also in consideration.

(c) Experimental Design

The obtained rats were randomly divided into 4 groups (20 each). G 1 was left without any treatment as control group. The other groups (2, 3 & 4) were exposed to lead acetate in drinking water at a concentration of 30 mg/l according to Sujatha *et al.* (2011) for 3 months. G2 was used as lead group. G3 was divided into three subgroup a, b and c was treated with catechin in drinking water at a concentration of 49 mg/l according to Miltonprabu &Thangapandiyan (2013) for 7 days after 1, 2 & 3 month after Pb exposure, respectively. G4 was also divided into

three subgroup a, b & c which treated with calcium disodium EDTA in a dose of 50 mg/kg body weight, IP for five days after 1, 2 & 3 month of lead exposure according to Flora *et al.* (2007a).

(d) Samples collection and preparation

Six rats were taken randomly for 3 times intervals post-treatment at 30, 60 and 90 days for negative control and positive controls, 37, 67 & 97 for rats treated with catechin and 35, 65 & 95 for rats treated with calcium sodium EDTA. Rats were anesthetized with ether and sacrificed for blood and tissues collection. Blood samples were collected from medial canthus of orbital cavity and heart of these rats in vacutainer tubes containing EDTA as anticoagulant for estimation of heavy metals. Brains were collected and washed with phosphate buffer 0.1M pH 7.4 and small pieces was fixed in formalin for histopathological examination

2- METHODS
(a) Preparation of samples for histopathological examination:

Small pieces of brain were quickly removed and fixed in 10% neutral buffered formalin and routinely processed. Then sections (7 µm) of different groups were mounted on slides and dried overnight at 37°C. Sections were dewaxed in xylene and hydrated in a graded series of alcohols and stained for hematoxylin and eosin for histological evaluation (Drury & Wallington, 1980).

(b) Estimation of heavy metals levels
i- Preparation of the samples

All glassware, pipette tips and plastics ware are rinsed with 25% HNO_3 to avoid metal contamination. Brain and blood samples were digested according to the method described by Cholak *et al.* (1971) as following: One g of each sample was placed in clean and dry 100 ml volumetric glass flask. 5 ml purified nitric acid was added. Samples were heated on hot plate at 70°C for 10 minutes gently until the appearance of brownish fumes then heating was continued till complete digestion which was noticed by the disappearance of the liquid color. All digested tissue samples were filtered with whatman filter papers. The obtained clear filtrate was kept in clean and dry bottles for analysis. All solutions and standards were prepared using bidistilled water.

ii- Measurement of lead, copper and iron

Lead is estimated by atomic absorption spectrophotometer, model 210 VGP. Copper and iron is estimated by Graphite atomic absorption spectrometer ZEEnit 700 P analytikjena (Germany).

(c) Statistical analysis

The data were expressed as mean±SE. The results were analyzed statistically using one-way analysis of variance with Tukey and Dunnett multiple comparison tests as a post-tests. These analyses were carried out using the computer SPSS program for windows, version 16.0. Differences between and among the groups were considered significant if $P ≤ 0.05$ (Snedecor *et al.*, 1989).

RESULTS:

- Clinical signs and histophathological findings: Observation of investigated rats in the all treated groups (2, 3 & 4) revealed no apparent clinical signs.

- Levels of Pb, Cu and Fe recorded in this study were summarized in tables 1-6.

- The histopathological changes observed were clarified in figures 1-20.

In the G4 (Lead + CaNa$_2$EDTA), brain of examined rats showed improvement in the pathalogical appearance of neurons in hippocampus and congestion in blood vessels at 1st month. At 2nd month showed very little changes in the neurons of brain.

Table 1: Blood lead level (ppm) in catechin and CaNa$_2$EDTA treated rats.

Animal groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	4.19 ± 0.347	5.19 ± 0.306	3.86 ± 0.370
G2	18.38 ± 1.07^{a}	24.57 ± 0.803^{a}	29.73 ± 0.763^{a}
G3	13.27 ± 0.526^{ab}	23.21 ± 1.09^{a}	15.43 ± 0.466^{ab}
G4	12.87 ± 0.523^{ab}	20.25 ± 0.520^{ab}	13.19 ± 0.846^{ab}

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4

Table 2: Brain lead level (ppm) in catechin and CaNa$_2$EDTA treated rats.

Animal groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	4.29 ± 0.150	3.65 ± 0.390	5.07 ± 0.141
G2	10.11 ± 0.881^{a}	20.25 ± 1.13^{a}	32.57 ± 1.32^{a}
G3	8.53 ± 0.824^{a}	13.73 ± 1.18^{ab}	19.79 ± 0.783^{ab}
G4	7.10 ± 0.011^{ab}	8.99 ± 0.820^{abc}	6.65 ± 0.481^{bc}

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4

Table 3: Blood copper level (ppm) in catechin and CaNa$_2$EDTA treated rats.

Animal groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	10.70 ± 0.777	10.03 ± 0.594	11.28 ± 0.460
G2	10.47 ± 0.384	8.52 ± 0.421	9.53 ± 0.621
G3	8.43 ± 0.630	14.77 ± 0.778^{ab}	12.84 ± 0.582^{b}
G4	14.31 ± 0.953^{abc}	11.09 ± 0.887^{c}	14.68 ± 0.936^{ab}

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4

Table 4: Brain copper level (ppm) in catechin and CaNa$_2$EDTA treated rats.

Animal groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	14.52 ± 0.550	14.93 ± 0.502	14.06 ± 0.259
G2	12.70 ± 0.296	8.94 ± 0.419^{a}	9.90 ± 0.669^{a}
G3	12.34 ± 0.876	13.65 ± 0.816^{b}	10.44 ± 0.323^{a}
G4	15.20 ± 0.425^{c}	12.81 ± 0.936^{b}	14.81 ± 0.622^{bc}

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4

Table 5: Blood iron level (ppm) in catechin and CaNa₂EDTA treated rats.

| Animal groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	453.87 ± 12.13	463.85 ± 10.22	452.12 ± 13.40
G2	545.9 ± 5.78[a]	464.67 ± 7.01	584.12 ± 11.25[a]
G3	366.29 ± 8.30[ab]	425.39 ± 7.71[b]	428.76 ± 17.4[4b]
G4	379.85 ± 10.58[ab]	409.50 ± 9.05[ab]	449.94 ± 17.37[b]

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4

Table 6: Brain iron level (ppm) in catechin and CaNa₂EDTA treated rats.

Exposed groups	Post- exposure time (months)		
	1st	2nd	3rd
G1	62.08 ± 2.53	62.42 ± 2.19	62.27 ± 2.03
G2	67.45 ± 2.81	40. 24 ± 1.00[a]	26.74 ± 1.97[a]
G3	61.70 ± 3.55	53.66 ± 1.03[ab]	40.61 ± 1.26[ab]
G4	70.68 ± 1.47	56.74 ± 0.995[b]	59.56 ± 1.56**bc**

a: Significant difference with G1   **b:** Significant difference with G2   **c:** Significant difference between G3 and G4
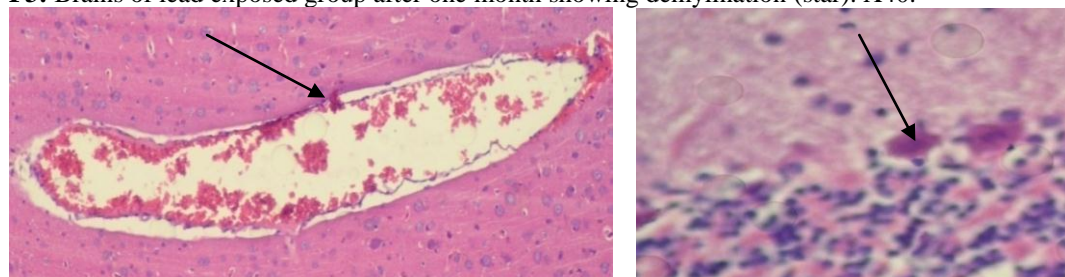
Histopathological examination:
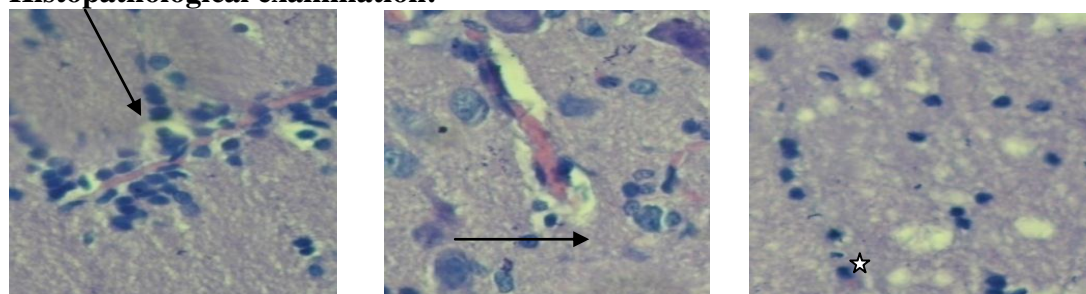
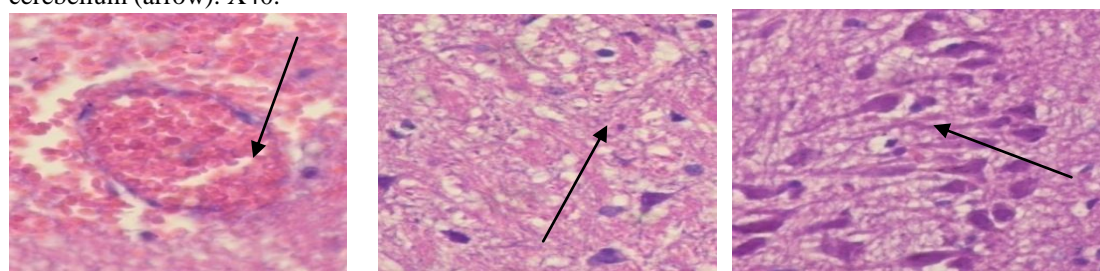**F1:** Brains of lead exposed group after one month showing perivascular cuff (arrow). X40.
F2: Brains of lead exposed group after 1 month showing neuronal degeneration and satelletosis demylination (arrow). X 40.
F3: Brains of lead exposed group after one month showing demylination (star). X40.

F4: Brains of lead exposed group after 1 month showing congestion in blood vessels (arrow). X10.
**F5:** Brain of lead exposed group after 2 month showing degeneration in purkinje cells in the cerebellum (arrow). X40.

F6: Brain of lead exposed group after 2 month showing perivascular hemorrhage and degeneration in blood vessels in cerebellum. X40.
**F7:** Brains of Pb exposed group after 2 month showing demylination in cerebral tissue.X40.
F8: Brain of Pb exposed group after 2 month showing degeneration of neurons and demylination in hippocampus. X40.
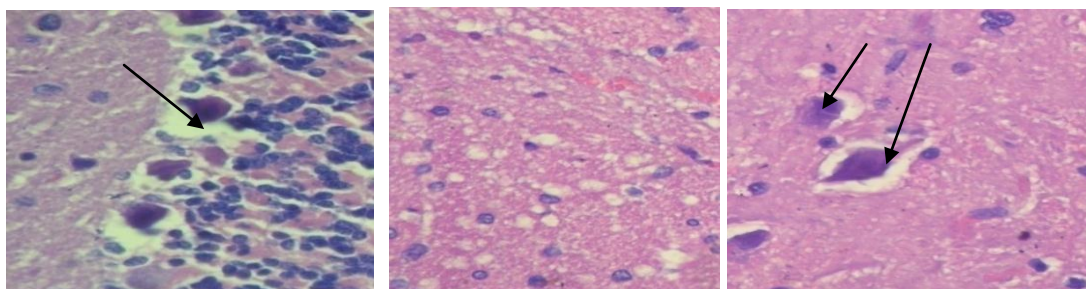
F9: Brains of Pb exposed group after 3 month showing degeneration of purkinje cells and congestion in cerebellum blood vessels (arrow). X40.

F10: Brains of catechin treated group after 1 month showing degeneration of neurons in gray matter. X40
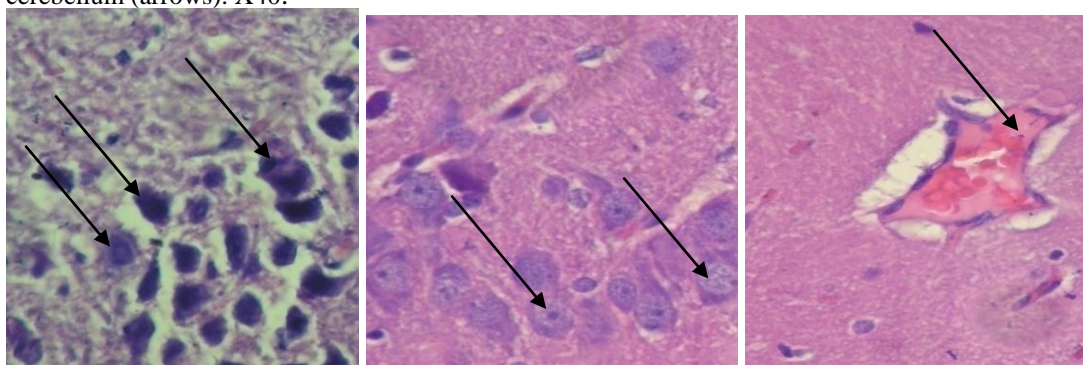
F11: Brains of catechin treated group after two month showing neuronal degeneration (arrows). X40.
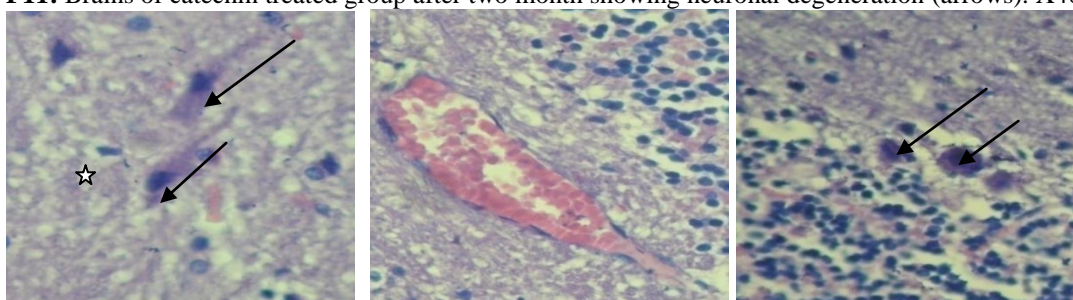
F12: Brain of catechin treated group after 3 month showing demylination (star) and neuronal degeneration (arrow). X40

F13: Brain of catechin treated group after 3 month showing neuronal degeneration and congestion. X40

F14: Brain of catechin treated group after 3 month showing degeneration in purkinje cells in the cerebellum (arrows). X40.

F15: Brains of catechin treated group after 3 month showing neuronal degeneration in the hippocampus (arrows). X40.

F16: Brains of Ca Na₂ EDTA treated group after one month showing normal neurons in the hippocampus (arrows). X40.

F17: Brains of Ca Na₂ EDTA treated group after one month showing congestion in blood vessels (arrow). X40.
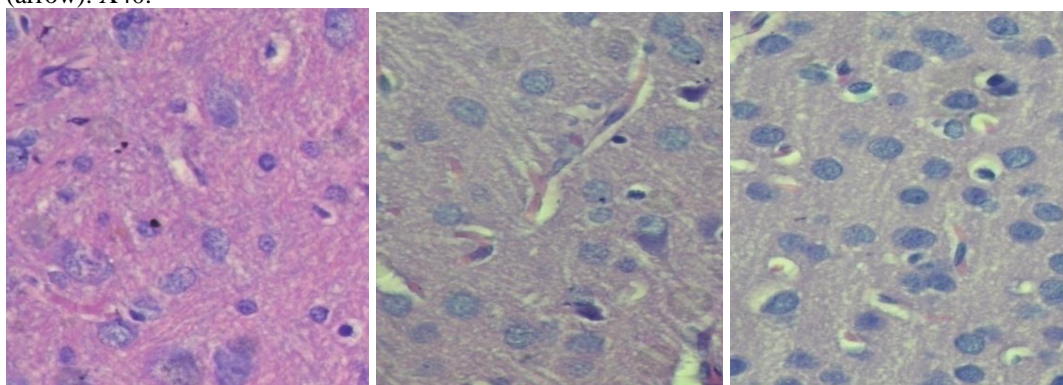
F18,19 & 20: Brain of Ca Na₂ EDTA treated group after one month showing normal neurons. X40.

DISCUSSION

Lead level in blood showed a significant increase all over the experiment period in G2 in comparison with G1 and in G3 all over the experiment period in comparison with control but significant decrease 1st and 3rd month when compared to G2. This was in agreement with Hamed *et al.* (2010) who reported that green tea co-administrated with Pb reduced Pb contents, increased antioxidant status in blood and brain tissue of Pb treated rats.. In G4 a significant increase all over the the experiment period in comparison with G1 but significant decrease all over the whole period of experiment when compared to G2. This was in agreement with Waters *et al.* (2001) who found that EDTA significantly increase Pb excrection in urine. Saxena & Flora (2004) concluded that $CaNa_2EDTA$ effectively reduce Pb concentration in blood, liver, and kidney copper content in brain tissue showed a significant decrease at 2nd and 3rd month in G2 in comparison with control. In G3 a significant decrease at 3rd month in comparison with G1but significant increase at 2nd month when compared to G2 and significant decrease at 1st and 3rd month when compared to G4. In G4 a significant increase at 2nd and 3rd month when compared to G2 and at 1st and 3rd month when compared to G3. These results contraindicated with Mehta & Flora (2001) who found that Cu concentration decreased by EDTA administration. However, administration of $CaNa_2EDTA$ alone in healthy dogs led to sustained urinary loss of Cu through mobilization and redistribution of the element from storage tissues (Ibim *et al.*, 1992).

Lead content in brain tissue showed a significant increase all over the whole period of experiment in G2 in comparison with G1. This was in agreement with Saxena & Flora (2004) who found that Pb exposure resulted in a significant increase in Pb concentration in blood, brain, liver, and kidneys. In G3 a significant increase all over the whole period of experiment in comparison with control but significant decrease at 2nd and 3rd month in comparison with G2 and significant increase at 2nd and 3rd month in comparison with G4. This was in agreement with Mehana *et al.* (2012) who found that Pb concentrations were significantly reduced in tissues of rats which received Pb combined with green tea extract and they suggest that the reduction of Pb concentrations in tissues of the rats treated with green tea extract may be due to its chelating property and thus decrease its gastrointestinal absorption. Moreover, the chelating agents form an insoluble complex with Pb and remove it from Pb-burdened tissues (Patrick, 2006a). In G4 a significant increase at 1st and 2nd month in comparison with G1 but significant decrease all over the experiment period in comparison with G2 and significant decrease at 2nd and 3rd month in comparison with G3. These results are contraindicated with Saxena & Flora (2004) who found that inefficiency of EDTA to decrease brain Pb. In this aspect, $CaNa_2EDTA$ was not efficacious in reducing brain or bone Pb levels, although brain levels of labile ^{204}Pb tracer were significantly reduced after 5 days of chelation (Seaton *et al.*, 1999). Although, Flora *et al.* (2007b) found that administration of $CaNa_2EDTA$ marginally depleted the brain Pb concentration.

Level of Cu in blood showed no significant difference in G2 in comparison with G1. This was contraindicated with Flora *et al.* (1998) who found that Pb exposure causes depletion in blood Cu level. A significant increase at 2nd and 3rd month in G3 when compared to G2 but showed a significant decrease at 1st month and significant increase at 2nd month when compared

to G4. This was in agreement with Cooper *et al.* (2005) who found that green tea promoted the absorption of Cu. In G4, Cu content showed a significant increase at 1st and 3rd month in comparison with control and G2 and at 1st month when compared to G3. This in agreement with Patra & Swarup (2005) who found that treatment with EDTA alone showed improvement in blood Cu level but Flora *et al.* (1998) found that $CaNa_2EDTA$ had no effect on blood Cu concentration. In this aspect, plasma Cu level was significantly higher compared with the G1and correlated positively with Pb concentrations (Kasperczyk *et al.*, 2012). The results were explained by Kasperczyk *et al.* (2004) who mentioned that Pb exposure was associated with an elevated activity of Cu/Zn-SOD in both serum and erythrocytes. Therefore, an increase in the Cu level, may be caused by increased Cu/Zn-SOD activity. This enzyme is part of the antioxidant defence system and its activity may be elevated by Pb (Kasperczyk *et al.*, 2005). The increase in plasma Cu levels may also be caused by competitive displacement of the metal from tissues by Pb ions and the increased bioavailability of displaced Cu may induce ROS generation via the Fenton reaction and contribute to oxidative stress enhancement (Qian *et al.*, 2005). Although binding constants of Cu^{2+} ions with EDTA is higher than that of Pb and Cu would be lost during chelation therapy. Waters *et al.* (2001) showed that no significant increase in Cu excrection occur after EDTA infusion despite its very high binding constants because 95% of Cu in plasma is bound to ceruloplasmin (Burtis & Ashwood, 1994). The Cu atoms are tightly bound and not in equilibrium with surrounding aqueous medium therefore on significant increase in Cu excretion following EDTA indicate that this chelating agent is unable to effect dissociation of Cu from ceruloplasmin (Waters *et al.*, 2001).

Iron in blood showed significant increase in G2 at 1st and 3rd month in comparison to control. This was in agreement with Taha *et al.* (2013) who found a significant increase in serum iron level after Pb exposure and suggest that progressive destruction of RBCs and increasing fragility of RBCs membrane may be a cause of increasing iron level. Oxidative stress also causes release of iron from proteins resulting in increased non-protein-bound iron concentrations (Benov, 2001).

Level of iron in G3 showed a significant decrease at 1st month in comparison to control and all over the whole period of experiment when compared to G2. This was in agreement with Samman *et al.* (2001) found a reduction in non-heme iron absorption. Iron induced malondialdehyde production and DNA damage were significantly reduced in Jurkat T cells grown in media supplemented with green tea extract, suggesting that catechins have a direct affinity for iron (Erba *et al.*, 1999). The phenolic monomers, polyphenols, and tannins that are found in tea are thought to interfere with iron absorption by forming insoluble complexes in the gastrointestinal lumen, thereby lowering the bioavailability of iron (Disler *et al.*, 1974). G4 showed significant decrease at 1st and 2nd month in comparison to control also significant decrease all over the experiment period in comparison to G2.

Iron level in brain tissue showed a significant decrease at 2nd and 3rd month in G2 in comparison with G1. This was in agreement with Kim *et al.* (2003) who found that Pb significantly decreases hemoglobin, serum iron levels and transferrin saturation and dietary iron

intake was inversely associated with zinc protoporphyrin. (Mudipalli, 2007) found that Pb inhibited transferrin endocytosis and iron transport across the cell membrane of reticulocytes. Lead has a high affinity for the side chains of at least five amino acids, which important for the uptake, transport, and utilization of iron. These proteins include transferrin (Qian *et al.*, 1997), mucin (Conrad *et al.*, 1991), mobilferrin (Conrad *et al.,* 1990), Divalent metal transporter 1 (DMT1) (Gunshin *et al.*, 1997), hemoglobin (Barltrop & Smith, 1972) and enzymes of heme synthesis (Goldberg, 1972). In a state where iron levels are inadequate, Pb may disrupt proper iron utilization and cause iron deficiency (Kwong *et al.*, 2004). In G3 a significant decrease at 2nd and 3rd month in comparison with control group but significant increase at 2nd and 3rd month when compared to G2 and decrease at 3rd month when compared to G4. Similarly, catechins treatment causes a decrease in iron absorption from the digestive tract (Crespy & Williamson, 2004). In consequence, low level of iron ions causes the diminution of free radicals generation and reactions with integral compounds of cells (Zapora *et al.*, 2009). In G4 Iron level was significantly increased at 2nd and 3rd month when compared to G2 and at 3rd month when compared to G3.

Hisopathological examination of brain tissues in Pb exposed rats after 1st month revealed perivascular cuff, neuronal degeneration and satelletosis demylination and congestion in blood vessels but at 2nd and 3rd month, there was sever degeneration of purkinje cells and perivascular hemorrhage and degeneration in blood vessels in cerebellum, demylination of cerebral tissue and degeneration of neurons and demylination in hippocampus. This was in agreement with Khalaf *et al.* (2012) who observed oedema in the hippocampus and between the cells associated with focal gliosis in the cerebrum, vacuolization in cerebellum and neuronal degeneration and gliosis in medulla oblongata. In consistence, Amal & Mona (2009) found that that brain of rats received 100 mg/L Pb acetate in drinking water for 3–6 weeks, showed pyknosis of neurons associated with focal gliosis and focal cerebral hemorrhage. Also, Deveci (2006) observed degeneration in some of neuron cells and dilatation in the lumen of the blood vessels in brain of rats treated with Pb acetate at a dose of 500 ppm in their drinking water for 60 days. In addition, treatment of rats with Pb at dose level of 50 mg/kg b.wt, caused degeneration of neurons in cerebrum (Sidhu & Nehru, 2004). In the present study, co administration of catechin with Pb acetate caused demylination and congestion in white matter and degeneration of neurons in gray matter at 1st month. At 2nd month brain tissue showing neuronal degeneration , demylination, congestion nd degeneration of purkinje cells in cerebellum. At 3rd month showing neuronal degeneration in hippocampus. These pathological observation in contrast with finding of Khalaf *et al.* (2012) who observed improvement of the histopathological alteration in brain compartment in rats which treated with green tea extract. Moreover, Amal & Mona (2009) found that brain of rats treated with Pb and mixture of vit. C, vit. A, vit. E & selenium for 3 or 6 weeks showed no histopathological changes except pyknosis of some neurons. However, co-treatmentof rats with CaNa$_2$EDTA showed improvement in brain with normal neuron and some congestion in blood vessels at 1st and 2nd month but after 3rd month brain showing normal neuron at hippocampus but demylination and congestion in white matter. In this aspect, Demougeot *et al.* (2004) observed

the protective effect of liposoluble iron chelator 2,2 dipyridyl (DP) in reducing histological damage in rats submitted to cerebral ischemia.

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تأثير الكاتشتن والاديتا أحادى الكالسيوم ثنائى الصوديوم على التسمم بالرصاص في الجرذان: دراسة متبقيات المعادن الثقيلة والتغيرات الهستوباثولوجية فى المخ.

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الملخص :

أُجريت هذه الدراسة لتقييم فعالية كلٍ من الكاتشن والاديتا في علاج التسمم بالرصاص على الجرذان من خلال دراسة متبقيات المعادن الثقيلة والتغيرات الهستوباثولوجية فى المخ. وقد تم استخدام ثمانين من ذكور الجرذان البيضاء قُسمت عشوائياً إلى ٤ مجموعات (٢٠ لكل منهم). تُركت المجموعة الأولى دون أي معامله كمجموعة ضابطه. تعرضت المجموعات الثلاث الأخرى (الثانيه، الثالثه والرابعه) لعنصرالرصاص فى صورة خلات في مياه الشرب بتركيز ٣٠ مجم/لتر لمدة ٣ أشهر. واستخدمت المجموعه الثانيه كمجموعة ضابطه موجبه. تم تقسيم المجموعة الثالثه إلى ثلاثة مجموعات فرعية A، B & C أُعطيت الكاتشن في مياه الشرب بتركيز ٤٩ مجم/لتر لمدة ٧ أيام بعد شهر و شهرين و ٣ أشهر من التعرض للرصاص على التوالي. تم تقسيم المجموعة الرابعه أيضا إلى ثلاثة مجموعات فرعية D، E & F وعوملت بالاديتا بجرعة ٥٠ مجم / كجم من وزن الجسم، عن طريق الحقن البريتونى لمدة خمسة أيام بعد شهر و شهرين و ٣ أشهر من التعرض للرصاص. أُخذت ستة من الفئران عشوائياً بعد ٣٠ و ٦٠ و ٩٠ يوماً من التعرض للرصاص من كل من المجموعه الاولى والثانيه وبعد ٣٧، ٦٧، ٩٧ يوماً من الفئران المعالجة بالكاتشين و ٣٥ و ٦٥ و ٩٥ يوماً من الفئران المعالجة بالاديتا. تم جمع عينات الدم وأنسجة المخ من جميع الجرذان.

وقد أظهرت النتائج مايلى: (١) كلا المركبين قلل من مستوى الرصاص فى المخ والدم ولكن الاديتا كان اكثر تاثيراً فى تقليل مستوى الرصاص فى المخ فى الشهرين الثانى والثالث. (٢) كلا المركبين رفع من مستوى النحاس فى الدم والمخ، وقد كانت حيث الاديتا الأكثر تاثيراً على انسجة المخ فى الشهرين الاول والثالث ولكن فى الدم كان الكاتشن مؤثراً فى الشهر الثانى بينما الاديتا كانت مؤثرة فى الشهر الاول. (٣) كلا المركبين قلل من مستوى الحديد فى الدم وزاد من مستواه فى المخ والاديتا كانت مؤثرة أكثر من الكاتشن فى الشهر الثالث. (٤) تعرض الفئران لخلات الرصاص أدت إلي تغيرات باثولوجية فى المجموعات الثلاث مقارنة بالمجموعات الضابط السلبية ولكن العلاج بمركب الاديتا أدى إلى إختفاء أو تخفيف بعض التغيرات الباثولوجية فى أنسجة المخ بصورة أفضل من الكاتشن.

الخلاصة: خَلُصت الدراسة إلي أن تعرض الجرذان للرصاص أدي إلي تغيرات باثولوجية في نسيج المخ اختلفت حسب مدة التعرض للرصاص. كما خلصت الدراسة إلي أن معالجة الجرذان بكل من الكاتشن والأديتا أدي إلي تحسن في المتغيرات السابقة بدرجات متفاوتة حسب مدة التعرض مع الأفضلية للكاتشن في تقليل مستوي الرصاص وتحسن التغيرات الباثولوجية.