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HEMATOLOGICAL AND BIOCHEMICAL IMPROVEMENT BY CATECHIN AND EDTA IN LEAD INTOXICATED RATS

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ABSTRACT

The present study was conducted to evaluate the efficacy of both Catechin and Calcium Disodium Ethylene diamine Tetraacetic Acid ($CaNa_2EDTA$) in treatment of long-term lead toxicity through assessment of some biochemical indices (hematological picture and biochemical parameters). Eighty male albino rats weighting 100-150 g of 10-12 weeks old were randomly divided into 4 groups (20 each). Group 1 was left without any treatment as negative control group. The other three 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 conrol group. G3 was divided into three subgroups A, B & C was treated with catechin in drinking water at a concentration of 49 mg/L for 7 days after 1, 2 and 3 month after Pb exposure, respectively. G4 was also divided into three subgroups (D, E & F) and treated through IP injection $CaNa_2EDTA$ in a dose of 50 mg/kg body weight, for 5 days after 1, 2 & 3 months of 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 $CaNa_2EDTA$. Rats were anesthetized with ether and sacrified for blood and tissues collection. Blood samples were collected in vacutainer tubes containing EDTA as anticoagulant for hematological pictures. Brain tissue samples were collected from each rat for the subsequent biochemical parameters (total protein, nitric oxide, lipid peroxidation, glutathione, superoxide dismutase, catalase, glucose 6 phosphatase dehydratase and, acetylcholinestrase activity). The results revealed that administration of catechin or $CaNa_2EDTA$ can minimize any toxic effects on hematological picture & biochemical parameters. Catechin was more effective than $CaNa_2EDTA$ in improvement of Hb concentration, HCT %, MCV, MCH values and NO**,** LPO parameters but $CaNa_2EDTA$ was more effective than Catechin in improvement of WBCs, lymphocyte, monocyte count and GSH, SOD, CAT activities.

Key Words: Hematological, Biochemical, Catechin, EDTA, Lead and Rats**.**

INTRODUCTION

In recent years, the level of heavy metals, particularly lead (Pb) has increased in air, water and soil in both urban and peri-urban areas (Gupta, 2007). Heavy metals induce toxic effects on different systems and apparatuses. Furthermore, because of their long half-life, heavy metals also induce accumulation phenomena, which in turn produce increase of their concentration in blood and tissues. Among heavy metals, Pb represents the main environmental pollutant is a non essential toxic heavy metal widely distributed in the environment and causes neurological impairment (Soong *et al*., 1999). The main sources of environmental Pb are from leaded gasoline, lead shots orbullets, soil, dust, toys, lead acid batteries, cosmeticsand paints (Thuppil and Tannir, 2013).Lead directly affects the hematopoietic system through restraining the synthesis of hemoglobin (Hb) by inhibiting three vital enzymesin pathway of heme synthesis, δ-aminolevulinic acid dehydratase, aminolevulinic acid synthetase, and the mitochondrial enzyme ferrochelatas that catalyzes the insertion of iron into protoporphyrin to form heme (Piomelli, 2002). It also reduces the life span of circulating erythrocytes by increasing the fragility of cell membranes these two processes leads to anemia (Guidotti *et al.*, 2008). In more advanced cases of Pb toxicity, absolute neutrophilia, leukocytosis (with shifting to left),

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eosinopenia, and monocytopenia have been reported (Xintaras, 1992).

Lead induced oxidative stress by increased the levels of lipid peroxidation (LPO) or decrease in antioxidant defence mechanism (Bokara *et al.,* 2008). Pb shows electron sharing capability that results in the formation of covalent attachments. These attachments are formed between the Pb moiety and sulfhydryl (-SH) groups present in antioxidant enzymes, which are the most susceptible targets for Pb and which eventually get inactivated (Flora *et al.*, 2012). Pb binds exclusively to the –SH group, which decreases the Glutathione (GSH) levels and can interfere with the antioxidant activity of GSH (Bechara, 2004) and decrease Catalase (CAT) ,Superoxide dismutase (SOD) enzyme commonly found in living tissue (Patil *et al.*, 2006).

Catechin (Green tea) is a free radical scavenger and can scavenge both hydroxyl and superoxide radicals as well as lipid free radicals and peroxyl radicals (Sutherland *et al.,* 2006). The health-promoting effects of green tea are mainly attributed to its polyphenol content (Khan and Mukhtar, 2007). The major tea catechins are (-)-epigallocatechin-3-gallate, (-)-epicatechin-3-gallate, (-)- epigallocatechin, (-)-epicatechin, (+)-gallocatechin, and (+)-catechin (Kaushik *et al.*, 2011). Moreover, green tea extracts and its isolated constituents are effective in preventing oxidative stress (Babu *et al.*, 2006) and neurological problems (Unno *et al.*, 2007). Intake of green tea extracts also increases the activity of SOD in serum and the expression of CAT in the aorta; these enzymes are implicated in cellular protection against reactive oxygen species (ROS) (Skrzydlewska *et al.*, 2002).

Calcium Disodium Ethylene diamine Tetraacetic Acid ($CaNa_2EDTA$) is the most commonly used chelating agent. It is a derivative of ethylenediaminete traacetic 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 and Flora, 2005).

Aim of the study to evaluate the protective effects of both Catechin and Calcium Disodium Ethylene diamine Tetraacetic Acid ($CaNa_2EDTA$) in treatment of long-term lead toxicity through assessment of some biochemical indices (hematological picture and biochemical parameters).

MATERIALS AND METHODS:

Chemicals:-

1- Catechin, ($C_{15}H_{14}O_)$ and 98% purity was obtained from Sigma chemicals Co., USA.

2- 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.

3- Lead acetate $[Pb(CH_3COO)_2.3H_2O]$ with molecular weight of 379.33 was obtained from EL Nasr Pharmaceutical Chemicals Co., Egypt.

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.

Experimental Design:

The obtained rats were randomly divided into 4 groups (20 each). Group 1 was left without any treatment as negative control. The other three groups (G2, G3 and G4) 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 positive conrol group. G3 was divided into three subgroup (A, B and C)and treated with catechin in drinking water at a concentration of 49 mg/L according to (Miltonprabu and Thangapandiyan, 2013) for 7 days after 1, 2 and 3 months post Pb exposure, respectively. G4 was also divided into three subgroup (D, E and F) and treated with calcium disodium EDTA in a dose of 50 mg/kg body weight, IP for 5 days after 1, 2 & 3 months post lead exposure according to (Flora *et al.*, 2007).

Time schedule for samples collection and preparation:

Samples were collected from six rats taken randomly 3 times with intervals at 30, 60 and 90 days from negative and positive control 37, 67, 97 from rats treated with catechin and 35, 65 and 95 from rats treated samples were 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 of these rats in vacutainer tubes containing anticoagulant for hematological pictures.

Brain collected from each rat, washed with phosphate buffer 0.1M pH 7.4 and used for preparation of cytosol. 10% homogenate of brain samples were prepared by homogenization of 250 mg of each tissue in 2.5 ml (0.1 M) phosphate buffer (pH 7.4) using homogenizer (IKA Yellow line DI 18 Disperser, Germany). The homogenates were centrifuged at 6,000 rpm for 1 hour at 4 °C and the supernatant cytosols were kept frozen at -20 °C for the subsequent biochemical parameters (total protein, nitric oxide, lipid peroxidation, glutathione, superoxide dismutase,

catalase, glucose 6 phosphatase dehydratase, acetylcholinestrase activity).

Hematological parameters:
Complet blood picture was performed by Haematological Analizar (Media Serve, Exigo Haematology Analizar) at Pathology and Clínical Pathology Department, Faculty of Veterinary, Assiut University.

Biochemical parameters:
1-Assessment of total protein:
Total protein concentration in brain cytosol was determined by the method of Lowry *et al.* (1951).

2- Assessment of NO:
NO was measured as nitrite concentration in brain according to the method of Ding *et al.* (1988).

3-Assessment of LPO:
Product of LPO as TBARS in brain was estimated according to the method of ohkawa *et al.* (1979).

4-Assessment of GSH:
GSH content in brain was determined using the method of Beutler *et al.* (1963).

5- Assessment of SOD activity:
The activity of SOD in tissue cytosols was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of Misra and Fridovich (1972).

6- Assessment of CAT activity:
Activity of CAT in tissue cytosols was determined basing on its ability to decompose H_2O_2 according to Luck (1963).

7- Assessment of G6PD activity:
The activity of G6PD in tissue cytosols was determined according to its ability to reduce NADP according to the method of Haghighi *et al.* (1998).

8- Assessment of AChE activity:
AChE enzyme activity was estimated according the method of Ellman *et al.* (1961).

Statistical analysis:
The data was 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 prism program for windows, version 6.0 (Graph pad software Inc., San Diego, California, USA) and the computer SPSS program for windows, version 16.0. Differences between and among the groups were considered significant if $P \leq 0.05$.

RESULTS

Hematological parameters:
In the present study, RBCs count showed significant decrease in G2 after three months post exposure in comparison with the negative control. However, there is significant increase in RBC count after three months in G3 and after 2nd and 3rd month in G4 in comparison with G2 (Table 1).

A significant decrease of Hb concentration was recorded during the whole period of the experiment in G2 when compared with the control group. In G3, Hb level showed a significant increase all over the the experiment period in comparison with G2.While Hb level was significantly increased after the end of 2nd and 3rd month in G4 when compared with G2 (Table 2).

Haematocrit (HCT) percent was significantly increased in G3 during all the experiment period and in G4 after the end of 2nd month in comparison with G2 (Table3).

MCV values was significantly increased in G3 after three months although it showed a significant decrease after 2nd month in G4 when compared with G2 (Table 4).

MCH showed a significant increase in G3 after 3rd month when compared with G2 but it showed a significant\decrease after 2nd and 3rd month in G4when compared with the negative one (Table5).

In the present study, G3 showed significant decrease in WBC count after 1st and 2nd month and significantly increased after three months when compared with G2. In G4, a significant increase in WBC count was observed after 1st and 2nd month in comparison with control group and a significant increase all over the experiment period when compared with G2 and after 1st and 2nd month of treatment when compared with G3 (Table 6).

Lymphocyte count was significantly increased after three month In G3 and after 2nd and 3rd month in G4 when compared with G2 (Table7). However, granulocytes in G3 significantly decreased after 1st month and increased after 3rd month when compared with G2, while in G4, granulocyte count significantly decreased after 1st month and increased after 2nd and 3rd month when compared with G2 (Table 8). In addition, the present study revealed a significant increase in monocyte count after three month in G3 and after 1st and 2nd month when compared with G2 (Table 9).

Biochemical parameters:

In the present study, nitric oxide and lipid peroxidation levels in the brain tissue showed a significant increase in G2 (positive control) in comparison with negative control all over the experiment period. NO and LPO levels showed a significant decrease all over the experiment period in G3 when compared with G2. However, NO and LPO levels in G4 showed a significant decrease after 2 and 3 months of treatment (Table 10 and 11).

Data of the present study showed that GSH level in the brain tissue was significantly decreased after 1st and 2nd month in G2 in comparison to negative control. G3 and G4 showed significant increase in the levels of GSH all over the experiment period in comparison with G1 and G2 but G4 showed a significant increase in values of GSH after three months in comparison with G3 (Table 12).

In the present study SOD activity in brain tissue showed a significant increase in G3 after 1st and 3rd month in comparison with G1 and all over the experiment period when compared with G2. Also, in G4 a significant increase in SOD activity was found after 1st month in comparison with G1 and all over the experiment period when compared with G2 and after 1st month when compared with G3 (Table 13).

In the present study, the activity of CAT in brain tissue showed no significant change in G2.

G4 showed significant increase in CAT activity was found after 1st and 2nd month in comparison with G1 and all over the experiment period when compared with G2 and after 1st month when compared to G3 (Table 14).

The activity of G6PD in brain showed no significant change in G2 while it was significantly increased post 1st and 2nd month in G3 and G4 in comparison with G1 and G2 but G4 showed a significant increase in values of G6PD activity post 1st month in comparison with G3(Table 15).

AChE enzyme activity in brain tissue showed a significant increase in G3 after 2nd and 3rd month when compared with G2 and all over the experiment period in G4 in comparison with G2 and post 1st month when compared to G3 (Table 16).

Table 1: Effect of catechin and EDTA in RBC_S count (million/mm^3) of lead intoxicated rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	7.31 ± 0.549	7.17 ± 0.553	7.09 ± 0.513
G2	6.83 ± 0.366	6.59 ± 0.336	5.26 ± 0.149a
G3	7.51 ± 0.097	8.06 ± 0.163	8.26 ± 0.318b
G4	7.82 ± 0.134	8.65 ± 0.489b	7.63 ± 0.265b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 2: Effect of catechin and EDTA in Hb values (g/dl) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	14.06 ± 0.440	13.66 ± 0.185	14.16 ± 0.366
G2	11.33 ± 0.517a	11.93 ± 0.233a	10.46 ± 0.284a
G3	13.50 ± 0.35lb	14.16 ± 0.272b	14.23 ± 0.578b
G4	13.10 ± 0.305	14.80 ± 0.500b	12.96 ± 0.742b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 3: Effect of catechin and EDTA in HCT% of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	43.10 ± 0.351	41.56 ± 0.721	43.96 ± 0.218
G2	35.03 ± 0.517a	34 ± 0.305a	32.50 ± 0.550a
G3	39.10 ± 0.435abc	38.96 ± 0.233ab	38.90 ± 0.200abc
G4	36.20 ± 0.650ac	37.76 ± 0.54ab	34.53 ± 0.902ac

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 4: Effect of catechin and EDTA in MCV (u/mm^3) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	54.56 ± 0.120	52.56 ± 0.497	53.43 ± 0.523
G2	49.53 ± 0.260a	49 ± 0.503a	45.90 ± 0.709a
G3	50.80 ± 0.608a	49.23 ± 0.924ac	49.26 ± 0.523abc
G4	48.63 ± 1.28a	43.50 ± 0.838abc	45.20 ± 0.351ac

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 5: Effect of catechin and EDTA in MCH (pg) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	19.03 ± 0.366	18.90 ± 0.378	18.76 ± 0.317
G2	17.10 ± 0.378a	16.63 ± 0.284a	15.70 ± 0.472a
G3	18.13 ± 0.328	17.76 ± 0.266	17.90 ± 0.115b
G4	17.86 ± 0.497	17.06 ± 0.296a	16.60 ± 0.264a

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 6: Effect of catechin and EDTA in WBCs counts (thousand/mm^3) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	7.63 ± 0.375	7.86 ± 0.348	8.06 ± 0.120
G2	9.33 ± 0.145a	6.33 ± 0.176	3.73 ± 0.440a
G3	6.00 ± 0.115abc	4.26 ± 0.338abc	7.10 ± 0.404b
G4	11.50 ± 0.556abc	11.26 ± 0.664abc	7.40 ± 0.585b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 7: Effect of catechin and EDTA in Lymphocyte counts (thousand/mm3) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	5.20 ± 0.115	5.06 ± 0.088	5.63 ± 0.088
G2	6.03 ± 0.202a	3.73 ± 0.218	2.73 ± 0.233a
G3	5.26 ± 0.202c	2.63 ± 0.176ac	4.70 ± 0.360b
G4	6.43 ± 0.176ac	8.76 ± 0.666abc	5.66 ± 0.317b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 8: Effect of catechin and EDTA in Granulocyte counts (thousand/mm3) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	1.33 ± 0.088	1.40 ± 0.115	1.46 ± 0.088
G2	2.60 ± 0.057a	0.866 ± 0.066a	0.800 ± 0.057a
G3	1.40 ± 0.115bc	1.10 ± 0.057	2.13 ± 0.033abc
G4	2.03 ± 0.145abc	1.26 ± 0.066b	1.23 ± 0.120bc

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 9: Effect of catechin and EDTA in Monocyte count (thousand /mm^3) of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	0.500 ± 0.057	0.400 ± 0.057	0.566 ± 0.066
G2	0.466 ± 0.033	0.366 ± 0.033	0.200 ± 0.057a
G3	0.600 ± 0.057c	0.533 ± 0.033c	0.466 ± 0.066b
G4	0.900 ± 0.057abc	1.23 ± 0.088abc	0.433 ± 0.033

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 10: Effect of catechin and EDTA in nitric oxide concentration (nmol/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	10.45 ± 0.386	11.12 ± 0.389	11.45 ± 0.386
G2	18.20 ± 0.275a	17.26 ± 0.754a	22.82 ± 1.78a
G3	14.27 ± 0.410ab	9.56 ± 0.427b	3.61 ± 0.102ab
G4	16.60 ± 1.09a	12.69 ± 1.23b	6.18 ± 0.370ab

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 11: Effect of catechin and EDTA in lipid peroxide concentration (nmol/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	0.094 ± 0.009	0.091 ± 0.005	0.097 ± 0.007
G2	1.44 ± 0.080a	1.85 ± 0.079a	2.18 ± 0.080a
G3	1.16 ± 0.059ab	1.37 ± 0.088ab	1.70 ± 0.070ab
G4	1.18 ± 0.057a	1.26 ± 0.017ab	1.56 ± 0.064ab

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 12: Effect of catechin and $CaNa_2EDTA$ in GSH content (Micro nmol/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	0.260 ± 0.023	0.250 ± 0.025	0.276 ± 0.021
G2	0.165 ± 0.019a	0.139 ± 0.015a	0.222 ± 0.022
G3	1.50 ± 0.012ab	0.887 ± 0.036ab	0.686 ± 0.099abc
G4	1.49 ± 0.012ab	0.946 ± 0.013ab	0.954 ± 0.053abc

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 13: Effect of catechin and EDTA in SOD activity (unit/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	2.01 ± 0.029	2.09 ± 0.008	2.02 ± 0.011
G2	1.01 ± 0.069a	1.43 ± 0.123	1.00 ± 0.095a
G3	3.72 ± 0.207abc	2.76 ± 0.160b	2.51 ± 0.090ab
G4	5.02 ± 0.305abc	2.77 ±0.271b	2.36 ± 0.154b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 14: Effect of catechin and EDTA in CAT activity (unit/ min/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	0.105 ± 0.013	0.106 ±0.009	0.105 ± 0.010
G2	0.133 ± 0.014	0.107 ± 0.017	0.063 ± 0.005
G3	0.128 ± 0.007c	0.178 ± 0.012	0.096 ± 0.005
G4	0.237 ± 0.021abc	0.193 ± 0.020ab	0.140 ± 0.019b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 15: Effect of catechin and $CaNa_2EDTA$ in G6PD activity (unit/mg protein) in brain cytosol of lead exposed rats (n=6).

Exposed Groups	Post-exposure time (months)		
	1st	2nd	3rd
G1	5.61 ± 0.563	5.70 ± 0.519	5.01 ± 0.858
G2	6.71 ± 0.475	4.47 ± 0.255	6.69 ± 0.666
G3	11.02 ± 0.696abc	15.31 ± 0.521ab	7.56 ± 0.509
G4	17.72 ± 1.31abc	13.79 ± 1.31ab	7.70 ± 0.557

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

Table 16: Effect of catechin and EDTA in acetylcholinesterase activity (mmol/min/mg protein) in brain cytosol of lead exposed rats (n=6).

ExposedGroups	Post-exposure time (months)		
	1st	2nd	3rd
G1	1.37 ± 0.100	1.27 ± 0.100	1.31 ± 0.094
G2	0.756 ± 0.026a	0.483 ± 0.028a	0.557 ± 0.030a
G3	1.00 ± 0.072c	1.54 ± 0.055b	1.26 ± 0.098b
G4	1.66 ± 0.161bc	1.55 ± 0.134b	1.30 ± 0.129b

All values was expressed as (meen ±SE)
a significant at p≤ .05 in comparison with control group
b indicates significant difference with G2
c indicates significant difference between G3 and G4

DISCUSSION

Pb is an environmentally persistent toxin that lead to neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological disorders (Patrick, 2006).

Development of anemia in lead toxicity may be attributed to the decreased red blood cell survival because of the increased membrane fragility, reduced RBCs count, decreased hemoglobin production, or summation of all these factors (Rio *et al.*, 2001). In the present study, RBCs count showed significant decrease in G2 after exposure for three months in comparison with the control group which was in agreement with Goneneci *et al.* (2008). However, RBCs count in G3 showed significant increase after three months in comparison with positive control. This result was in agreement with Miltonprabu and Thangapandiyan (2013) who found that epigallocatechin gallate (one of green tea catechins) improved altered hematological parameters by bringing them back to near normal levels. Also, there is significant increase in RBCs count in G4 after end the 2nd and 3rd month in comparison with G2. This finding was in agreement with Ashour *et al.* (2007) who found that CaNa2-EDTA exerted improvements in all hematological parameters and returned their levels to near those of controls.

A significant decrease in Hb concentration was recorded during the whole period of the experiment in G2 when compared with the control group. Similar result was obtained by Ibrahim *et al.* (2012). Induction of anaemia by Pb results from shortening of erythrocyte life span and an inhibition of Hb synthesis (Mudipalli, 2007). In G3, Hb level showed significant increase all over the experiment period in comparison with G2. This was in agreement with Gad and Zaghloul (2013) who found that Hb level was significantly increased in rats treated with green tea extract. Moreover it was significantly increased after 2nd and 3rdmonth of treatment in G4 when compared with G2. Similar result was recorded by Ashour *et al*. (2007) who found that treatment with $CaNa_2$-EDTA returned Hb values to near control values.

HCT percent was significantly increased in G3 in all over the experiment period when compared with G2 and after 2nd month in G4 in comparison with G2. In this aspect, Mladenović *et al.* (2014) reported that administration of epicatechin significantly increased HCT% in male Wistar albino rats. MCV and MCH values were significantly increased in G3 after three

months post exposure when compared with G2. This was in agreement with Gad and Zaghloul (2013) who found that hematological parameters were improved in rats treated with green tea.

In the present study, G3 showed increase at WBCs count after 3 months when compared with G2. Gad and Zaghloul (2013) found the same results in rats treated with green tea extract. In G4, a significant increase in WBC count was observed after 1st and 2nd month of exposure in comparison with G1 and during the whole experiment period when compared with G2 and after 1st and 2nd month when compared with G3. however, Flora *et al.* (1998) found that WBCs counts were not affected following $CaNa_2EDTA$ administration.

In G3 the NO level showed a significant increase after one month but decrease after three months in comparison to control group and all over the experiment period in comparison to G2. This was in agreement with Mandel *et al.* (2011) they found that green tea catechins (epigallocatechin gallate) inhibit NOS. Level of NO in G4 showed a significant increase after one month but it was significantly decreased after three months in comparison to G1 and after the 2nd and 3rd month in comparison to G2. These results were disagree with Flora *et al.* (2007) who found that Pb exposure increased NOS expression in brain, which could not be corrected by treatment with DMSA and/or $CaNa_2EDTA$.

LPO levels in brain showed significant increase in G3 all over the experimental period in comparison to G1 although it was significantly decreased all over the experimental period in comparison to G2. Skrzydlewska *et al.* (2002) mentioned that green tea extract can decrease lipid peroxidation in rat serum, liver and brain tissues. Moreover, catechins can also interact with phospholipid head groups, particularly with those containing hydroxyl groups, hence they may decrease the fluidity in the polar surface of phospholipid bilayer (Chen *et al.*, 2002). Level of LPO in G4 showed significant increase all over the experimental period in comparison to G1and showed decrease after 2nd and 3rd month in comparison to G2. In this aspect, Mehta and Flora (2001) found that hepatic LPO was significantly increased by EDTA administration.

Data of the present study indicated that GSH level in the brain tissue of G3 showed significant increase all over the experiment period in comparison with G1 and G2 and significant decrease after the 3rd month in comparison with G4. This was in agreement with Skrzydlewska *et al.* (2002) who revealed that green tea extract increases the activities of GPx and GR, and the content of GSH and diminishes the level of LPO in liver and CNS tissues. Level of GSH in G4 showed a significant increase all over the experimental period in comparison with G1, G2 and G3 (only after three months of exposure). These results were in agreement with Gopal *et al.* (2009) who reported that $CaNa_2EDTA$ administration caused significant increase in GSH in liver, kidney and muscle and disagree with (Mehta and Flora, 2001) who found that a significant decrease in hepatic GSH by EDTA administration.

SOD activity in brain tissue showed significant decrease after 1st and 3rd month in G2 when compared with control group. Khalaf *et al.* (2012) found that oral administration of lead acetate induced significant decrease in the activity of SOD in brain.

SOD activity was significantly increased after 1st and 3rd month in G3 in comparison with G1 and all over the experiment period when compared with G2. In this aspect, Al-Rejaie *et al.* (2012)stated that green tea supplementation bring back the SOD activity to normal in brain cells in stressed animals. A significant increase in SOD activity was found all over the experiment period in G4 when compared with G2 and after 1st month when compared to G3. These fiding indicated that Co- administration of $CaNa_2EDTA$ was more effective than catechin in improvement of the SOD activity.

In the present work, the activity of CAT in brain tissue showed no significant change in G2 and significantly decreased after 1st month in G3 in comparison with G4. These results disagreed with Antonio-García and Massó-Gonzalez (2008) who found that strong increase of catalase activity in the brain of rats exposed to 300 mg/L of lead during gestation and lactation and the CAT activity increased in Pb exposure due to formation of brain lipid hydroperoxides in Pb-intoxicated animals acts as a signal to maintain higher levels of catalase to enhance the triggering of the detoxification process for the metal. In our result CAT activity showed significant increase after 1st and 2nd month in G4 in comparison with G1 and all over the experiment period when compared to G2 and after 1st month when compared to G3.

The activity of G6PD in brain showed no significant change in G2. This was in agreement with Rogers *et al.* (1971) who found that G6PD activity was not changed after Pb intoxication. There was a significant increase in G6PD activity after 1st and 2nd month in G3 in comparison with G1 and G2. This was in agreement with Miltonprabu and Thangapandiyan (2013) who found a significant increase in G6PD activity in rats treated with epigallocatechin gallate. Our result revealed significant decrease in G3 after 1st month when compared to G4. These results in contrast with Pandiyan and Prabu (2014) who reported that Epigallocatechin gallate supplementation resulted in a significant decrease in G6PD activity. Finally, Pb can result in an increase or decrease in G6PD activity depending on the dose of

Pb, duration of Pb exposure, and magnitude of oxidative stress inside the cell (Gelman *et al.*, 1978).

Acetylcholinesterase activity in brain tissue showed significant decrease all over the whole period of the experiment in G2 in comparison with G1. This was in agreement with Reddy *et al*. (2003) who observed decrease in the activity of AChE after pbexpousure in both the cerebellum and hippocampus. Exposure to lead alters the release of neurotransmitter from presynaptic never endings. Spontaneous release of neurotransmitter is enhanced probably due to activation of protein kinases in the nerve endings (Sharma *et al*., 2014). In the present experiment, there is a significant increase AChE activity after 2nd and 3rdmonth in G3 when compared to G2. However, a significant decrease after 1st month was seen when compared to G4 in AChE activity. These results were in agreement with Rizvi and Zaid (2001) who reported that epicatechin caused an elevation in AChE activity in diabetic erythrocytes but it is incompetable with Kim *et al*. (2004) who reported that tea polyphenol administration inhibited AChE activity as compared to the control in a dose-dependent manner. In G4, a significant increase in AChE activity was observed along the experiment in comparison with G2 and after 1st month in comparison with G3. This was in agreement with Saxena and Flora (2004) they showed that treatment with $CaNa_2EDTA$ was successful in restoring altered AChE activity towards normal values.

In Conclusion, lead induced microcytic hypochromic anemia in lead treated rats. Administration of Catechin and $CaNa_2EDTA$ improved the hematological and biochemical parameters. Catechin was more effective than $CaNa_2EDTA$ in improvement of Hb, HCT %, MCV, MCH values and NO, LPO parameters, but $CaNa_2EDTA$ more effective than Catechin in improvement of WBCs, lymphocyte, monocyte count and GSH, SOD, CAT activities. AChE activity decreased in lead exposed group but increased in catechin and $CaNa_2EDTA$ treated groups.

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تحسين التغيرات الدموية والبيوكيميائيه بواسطة الكاتشين والاديتا في الفئران المتسممة بالرصاص

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أجريت هذه الدراسة لتقييم فعالية كل من الكاتشين والاديتا (CaNa2EDTA) في علاج تسمم الرصاص على المدى الطويل من خلال تقييم بعض المؤشرات الكيميائية الحيوية (الصورة الدموية والقياسات البيوكيميائية). اجريت الدراسه على عدد ثمانين من ذكور الجرذان البيضاء قسمت عشوائيا إلى ٤ مجموعات (٢٠ لكل منهم). تركت المجموعة الاولى دون أي معامله كمجموعة ضابطه سلبية. تعرضت المجموعات الثلاث الأخرى (الثانيه، الثالثه والرابعه) لعنصرالرصاص فى صورة خلات في مياه الشرب بتركيز ٣٠ مجم / لتر لمدة ٣ أشهر. وقد استخدمت المجموعه الثانيه كمجموعة ضابطه موجبه. تم تقسيم المجموعة الثالثه إلى ثلاثة مجموعات فرعية A، C&B اعطيت الكاتشين في مياه الشرب بتركيز ٤٩ ملجم / 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، F&E, وعوملت بالاديتا بجرعة ٥٠ ملغم / كغم من وزن الجسم، عن طريق الحقن البريتونى لمدة خمسة أيام بعد شهر و شهرين و ٣ أشهر من التعرض للرصاص. أخذت ست من الفئران عشوائيا بعد ٣٠ و ٦٠ و ٩٠ يوما من التعرض للرصاص من كل من المجموعه الاولى والثانيه وبعد ٣٧، ٦٧، ٩٧ يوما من الفئران المعالجة بالكاتشين و ٣٥ و ٦٥ و ٩٥ يوما من الفئران المعالجة بالاديتا. وتخديرها بالأثير، وذبحها لجمع عينات الدم والأنسجة. وقد تم جمع عينات الدم في أنابيب تحتوي على مانع التجلط لفحص صورة الدم و جمع أنسجة المخ من جميع الفئران للقياسات البيوكيميائية (البروتين الكلي، وأكسيد النيتريك، بيروكسيد الدهون، الجلوتاثيون، سوبر اكسيد ديسميوتاز، الكتاليز، الجلوكوز سداسى الفوسفاتيز والاستيل كولين استريز). واظهرت النتائج ان الكاتشين و الاديتا قد حسنتا من صورة الدم والقياسات البيوكيميائية وقللتا من التاثير السام على بعض مكونات الدم الخلويه والكيميائيه. وقد كان الكاتشين أكثر فعالية من الاديتا في تحسين مستوى الهيموجلوبين، وبعض قياسات صورة الدم الاخرى مثل HCTوMCV وMCH كما خفض من مستوى أكسيد النيتريك، والاكسده الفوقيه للدهون وكانت الاديتا أكثر فعالية من الكاتشين في تحسين عدد الكريات البيضاء والخلايا الليمفاوية، ووحيدة النواه وكذا زيادة مستوى نشاط، الجلوتاثيون، سوبر اكسيد ديسميوتيز، الكتاليز.