

Ameliorating Effects of Vitamin C against Acute Lead Toxicity in Albino Rabbits

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Abstract: The present work is devoted to investigate the possible protective and ameliorating effects of the administration of Vitamin C against lead-induced toxicity. Albino rabbits were subdivided into four groups. The first was a control group, the second received lead acetate in water as (12.5 mg / kg body weight) for 15 days, the third received the same lead acetate dose and supplemented with Vitamin C (40mg / kg body weight) for 15 days and the fourth received the same lead acetate dose for 15 days, then treated with the same Vitamin C dose for another 15 days after poisoning stoppage. The adverse effects of lead on blood, retina and some tissues have been demonstrated after 15 days of oral lead administration. The electroretinogram (ERG) a-and b-wave were significantly reduced below the normal values. These retinal changes did not recover completely 15 days after ceasing of lead intake, as the b-wave amplitude was still significantly low. The ERG alterations were correlated to retinal histological changes. The results indicated that exposure of animals to lead caused a significant increase in blood and tissues lead concentration when compared with control group. A number of biophysical parameters such as magnetic susceptibility, electrical conductivity, viscosity, and super oxide dismutase activity were estimated in blood as toxicity hazards evaluation. Treatment with Vitamin C, either combined with lead or administered later seemed to offer a marked improvement of the ERG parameters, histopathological, biochemical and biophysical findings. The ERG parameters were reversed towards the normal values, retinal cytoarchitecture was much ameliorated and the changes in blood parameters were significantly alleviated. The ability of Vitamin C to reduce lead toxicity may relate to its antioxidant actions via free radical scavenging mechanism. Supplementation of diets with Vitamin C may be recommended to improve the body burden of lead.

Key words: lead poisoning - ERG - vitamin C - antioxidant - hemoglobin.

INTRODUCTION

Lead is being a ubiquitous environmental contaminant due to its significant role in modern industry (Shalan *et al.*, 2005). However, both occupational and environmental exposures remain a serious problem in many developing and industrializing countries (Yucebilgic *et al.*, 2003). It has many undesired effects, including neurological (Moreira *et al.*, 2001, Soltaninejad *et al.*, 2003), behavioral (De Marco *et al.*, 2005 and Moreira *et al.*, 2001), immunological (Ercal *et al.*, 2000), renal (Loghman-Adham, 1997), hepatic and especially hematological dysfunctions (Patra and Swarup, 2004). Lead toxicity is closely related to its accumulation in certain tissues and its interference with the bioelements, whose role is critical for several physiological processes. About 99% of the lead present in the blood is bound to erythrocytes. They have a high affinity for lead and contain the majority of the lead found in the blood stream, which makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread lead to different organs of the body (Sivaprasad *et al.*, 2003).

Exposure to lead causes vision defects, in humans (Merigan and Weiss, 1980). Some of the disturbances of vision have a central origin, but there may also be retinal involvement in these disorders. Lead has been reported to affect selectively the rod photoreceptor component of the electroretinogram (ERG) (Fox and Sillman, 1979). Lead causes a reversible decrease in this component. Decreased amplitude of ERG b-wave has been correlated with the onset of lead toxicity in occupationally- exposed workers (Goyer, 1990). Accumulating evidences have shown that lead causes oxidative stress by inducing the generation of reactive

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oxygen species (ROS) and weakening the antioxidant defense system of cells (Flora, 2002). In general, ROS are reported to damage the polyunsaturated fatty acids of the membrane phospholipids of the cells causing impairments of cellular functions (Halliwell and Gutteridge, 1985 and Sies, 1991) by damaging cellular biomolecules.

Ascorbic acid (Vitamin C) has been studied extensively in modulating lead intoxication. Ascorbic acid is known to have number of beneficial effects against lead toxicity. It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead (Flora and Tandon, 1986). There has been considerable debate concerning the relationship between vitamin C nutritional status and heavy metal body burden in lead induced toxic effects. Early reports found that vitamin C might act as a possible chelator of lead, with similar potency to that of EDTA (Goyer and Cherion, 1979). In this context our attention was drawn to a report (Simon and Hudes, 1999) on a population based study in USA where blood levels of lead was shown to be related to low level of Vitamin C in the blood stream.

The aim of the present study was to investigate the impact of the combined administration of Vitamin C with lead-induced toxicities.

MATERIAL AND METHODS

Chemicals and Herbs:

Lead acetate was purchased from Merck (Darmstadt, Germany). Lignocaine hydrochloride and ketamine hydrochloride were purchased from Amoun (Pharmaceutical Company, Egypt). Glutaraldehyde was obtained from Boehringer-Ingelheim, Germany. Ascorbic acid (Vitamin C) was supplied from (Memphis Co. for Pharm. & Chemical Ind., Egypt).

Animals and Experimental Design:

Animals were used in accordance to the ARVO (Association for Research in Vision and Ophthalmology) statement for the use of Animals in Ophthalmic and Vision Research. The experiment was approved by the ethical committee. All through the experiment duration, the animals were housed in separate cages, fed standard laboratory food and allowed free access to water in room lightening with a 12 hour light-dark cycle in animal house of Research Institute of Ophthalmology.

Eighteen New Zealand albino rabbits weighing between 2.5 and 3 kg, aged two months, of both sexes were used for this study. The rabbits were divided into four groups: control group consists of 6 rabbits were received distilled water for 30 days. Group **I** consists of 6 rabbits were received oral lead acetate (in water with concentration of 12.5 mg / kg body weight) for 15 days, this group was subdivided into two subgroups: G_{IA} subjected to electroretinographic (ERG) and light microscopic (LM) examination, the other 3 rabbits (G_{IB}) left free untreated for another 15 days to study the possible recovery. Group **II** consists of 3 rabbits received daily Vitamin C with concentration of 40mg / kg body weight in combination with lead acetate for 15 days. Group **III** consists of 3 rabbits were received lead acetate for 15 days, and then treated with Vitamin C for another 15 days.

Ophthalmologic Examination:

Slit lamp and indirect ophthalmoscopic examinations were performed (by ophthalmologist) for all eyes prior to the study to exclude animals with media opacity or retinal damage.

Electrophysiological Tests:

Electroretinogram (ERG) using the Italian EREV 99 system (for recording and analysis by averaging) was performed before the study to establish baseline standards, 15 days and 30 days after. The animals were anesthetized intravenously using Lignocaine hydrochloride (5mg / kg) and ketamine hydrochloride (50mg / kg). The rabbits were then dark adapted for at least 30 min after pupillary dilatation. The active electrode was placed near the margin of the lower eyelid; the reference electrode was placed on the forehead and the earth electrode was clipped to the earlobe. Recording of combined response was carried out using white flash stimulus having frequency of 1 flash/second, energy of 2 joules and off background intensity. Amplitudes were measured from baseline to the lowest point of the negative peak for the a-wave and from the latter (or baseline, if absent) to the positive peak for the b-wave.

Histological Examination:

After the ERG tests, rabbits were sacrificed. The eyes were immediately enucleated, and injected with 4% glutaraldehyde. The retina were sliced into small pieces then fixed in phosphate buffered glutaraldehyde for

8 hours. After a buffer wash, retinas were dissected, post fixed in 1-33% osmium tetroxide, dehydrated in a series of graded ethanol and embedded in Epon. Semi thin sections were cut and stained with toluidine blue for examination by light microscope (Bancroft and Stevens, 1996).

Determination of Blood Lead Level:

Blood lead level was determined according to the method described in the Pye-unicum instruction manual, 1980; using a Pye-unicum SP 90 series atomic absorption spectrophotometer based on the method described earlier (Salvin, 1968).

Biophysical Parameters of Blood:

1-Determination of Hemoglobin Magnetic Susceptibility:

After the ERG tests, blood samples were collected by heparinated capillary tubes from ear vein in heparin containing tubes. Magnetic susceptibility of hemoglobin was measured using (Albert and Banerjee, 1975) method. The force exerted on the sample tube in the magnetic field is determined with a commercial semi micro-balance. This rests upon a mechanical stage, adjustable in two directions, which permits adjustment of the sample between the polar pieces. Thermal fluctuations of magnetic coils result in inhomogeneous magnetic fields which are compensated by a water-cooled Wiess magnet. The volume magnetic susceptibility is given by:

$$K_s = \frac{\delta S}{S} \frac{W}{\delta W} \frac{\rho}{d} K_w + \left[1 - \frac{\delta S}{S} \frac{W}{\delta W} \frac{\rho}{d} K_a \right] \quad (1)$$

Where K_s is the volume magnetic susceptibility, δS is the weight change of the sample in and outside the field, δW is the weight change of water in and outside the field, S is the weight of sample, W is the weight of water, ρ is the density of sample, d is the density of water, K_w is the volume magnetic susceptibility of water at the room temperature of the measurements and was calculated from:

$$K_w = - 0.72145 \times 10^{-6} - 0.000108 (t-20) \times 10^{-6} \quad (2)$$

K_a is the volume magnetic susceptibility of air which is 0.029×10^{-6}
Molar magnetic susceptibility is given from:

$$\text{Molar Mag. Sus.} = K_s \times \text{hemoglobin molecular weight} \quad (3)$$

2-Determination of Hemoglobin Electrical Conductivity:

Conductivity of hemoglobin solution was determined using a conductivity meter type digimeter L21/L21 C analytical auto temperature (Mignon-Germany) with a rod electrode in protective poly vinyl chloride tube temperature consistent up to 100°C. Measurements were performed at constant frequency (1500 Hz sine wave in the range of 0 - 200µS/cm). The conductivity meter was calibrated before measurements using a standard solution.

3-Determination of Tissue Lead Level:

Tissue specimens were obtained at the time of euthanization including, brain, bone (femur) and liver. Dried bone was crushed with acid wash glass/glass motor and pestle. The lead concentration of the various tissues was determined by atomic absorption with a graphite furnace utilizing modified methods of (Yeager *et al.*, 1971).

4-Determination of Hemoglobin Viscosity:

Ostwald capillary viscometer was used to measure the time flow at constant temperature (25°C) and at constant concentration (1.3×10^{-4} M) of hemoglobin. The viscosity of the hemoglobin solution was calculated using the following equation:

$$\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} \quad (4)$$

Where h , r and t are the viscosity, density and the time of flow of hemoglobin solution respectively. h_o , r_o and t_o are the corresponding quantities of water. This method measures the relative viscosity of the hemoglobin solution from which if unity is subtracted, the specific viscosity (h_{sp}) is given. The method is repeated for several concentrations and a relation between h_{sp} and C is plotted, the result will be a straight line its extrapolation gives the intrinsic viscosity.

5-Determination of Super Oxide Dismutase Activity:

Determination of super oxide dismutase (SOD) activity was carried out by a RANDOX kit package (McCord and Fridovich, 1969).

Statistical Analysis:

Amplitudes of a- and b-waves of ERG were expressed as the mean wave amplitude \pm SD microvolt (μV). Analysis of variance (ANOVA) and t-student test were performed to compare the responses between groups and within the same group. A post-hoc test was used to isolate significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

Electrophysiological Results:

ERG results are shown in Table (1) and Figs. (1, 2, and 3). Before the study, ERG recordings showed no significant differences between control group and the other groups ($P > 0.05$, ANOVA). In group I_A , Oral administration of lead for 15 days results in a significant decline in a-&b-wave amplitudes in a comparison with control group (Table 1 and Fig. 1). After stoppage of lead administration for another 15 days (group I_B), the value of a-wave amplitude was improved but still less than control eyes, while the b-wave amplitude was significantly decreased as compared to control group (Table 2 and Fig. 2).

Administration of Vitamin C in combination with lead (group II) improved ERG parameters but still lower than control eyes. Statistical analysis showed a significant difference in a-&b-wave amplitudes between G_{II} and control group, while they were significantly high in comparison with group I_A (Table 1 and Fig. 1). Also in G_{III} (eyes treated with Vitamin C) both a-&b-wave amplitudes were revealed slight improvement. When compared to control group a significant difference was found in a- or b- amplitude while there was no significant difference in a- or b-wave amplitude between G_{IB} and G_{III} (Table 1 and Fig. 2). Both a-wave and b-wave ERG amplitudes for all groups were considered in Fig. (3).

Histological Examinations:

Light microscopic examination of semi thin section from the rabbit retina of control group revealed normal histological appearance of retinal layers (Fig. 4). On the other hand, the histological examination of the retina of rabbits in G_{IA} which received lead acetate only, revealed edema of all layers of the retina and several distinct alterations. The cytoplasm of the pigment epithelium (PE) contained few vacuoles and densely stained granules. In the photoreceptor layer, the affection was remarkable; the outer segments were fragmented and disrupted while the inner segment appeared vacuolated. The damage of photoreceptors was extended to their nuclei in the outer nuclear layer which showed evident sign of pyknosis, karyorrhexis & karyolysis. The cells of the inner nuclear layer (INL) and ganglion cell layer (GC) appeared swollen. Some cells of INL showed halo of clear cytoplasm. Inner plexiform layer contained numerous glial cells (Fig. 5). The rabbits in G_{IB} revealed slight improvement of photoreceptor layer in addition, edema of the retina in animal toxicated with lead was subsided. However, Muller cells in the INL appeared dense (Fig. 6).

Histological examination of the retina of rabbits in G_{II} which received lead acetate in combination with vitamin C, showed edema of all retina layers. The outer segments of the photoreceptors appeared fragmented. Most nuclei of the outer nuclear layer were surrounded by a clear cytoplasm, but still not damaged. However, the cells of the inner nuclear layer appeared swollen or pale stained nuclei (Fig.7). On the other hand, rabbit's retina of G_{III} that received lead and then treated by vitamin C showed severe damage to the photoreceptor layer (Fig. 8).

Table (2) represents lead concentration in both blood and tissues of rabbits under normal conditions, after oral administration of lead acetate alone (G_{IA} and G_{IB}) or combined with Vitamin C (G_{II}) or treated with Vitamin C (G_{III}). Attention should be drawn to lead concentration in the treated groups either with or without continuity of intoxication as it is very clear that the blood concentration in those animals that received treatment after stoppage of lead intoxication; showed a dramatic decrease. No significant chelation treatment was happened in bone. Liver and kidney lead concentration, if the intoxication source is ceased, are reversibly decreased after Vitamin C treatment application.

Table (3) represents the biophysical parameters of blood for rabbits under normal conditions, after oral administration of lead acetate alone (G_{IA} and G_{IB}) or combined with Vitamin C (G_{II}) or treated with Vitamin C (G_{III}). Biophysical parameters values showed a very noticeable improvement after Vitamin C treatment application. Super Oxide Dismutase (SOD) activity tends to be normal after treatment application especially after lead poisoning stoppage.

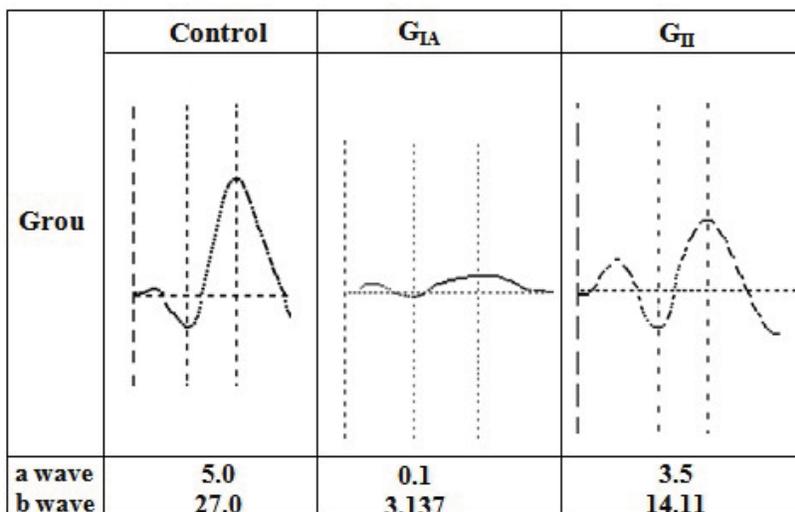


Fig. 1: ERG recording of selected rabbit eyes in groups G_{II} , G_{IA} and control group. ERG combined response demonstrated marked reduction of a- & b- wave amplitude in group G_{IA} . Both a- & b- wave amplitudes in group G_{II} were enhanced but still lesser than the normal value.

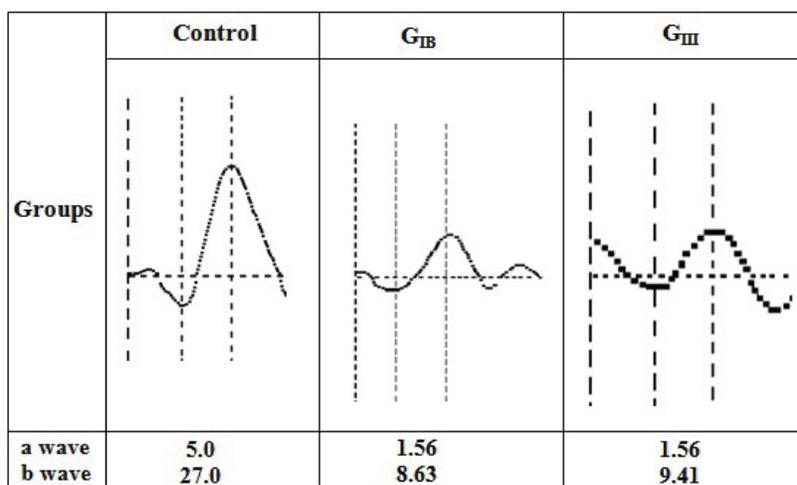


Fig. 2: ERG recording of selected rabbit eyes in groups G_{III} , G_{IB} and control group. ERG combined response demonstrated marked reduction of a- & b- wave amplitude in group G_{IB} and G_{II} .

Discussion:

Lead is common environmental toxin that is capable of causing numerous acute and chronic illnesses. It affects each and every organ and system in the body (Goyer, 1990). Neurotoxicity from lead exposure is of concern especially because lead at even very low concentrations can have profoundly detrimental neurological effects (Bellinger, 2004).

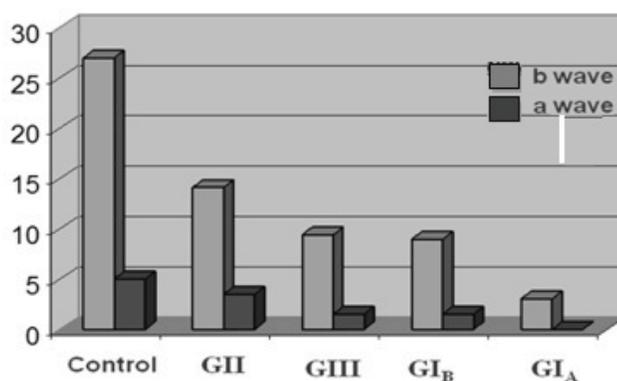


Fig. 3: The mean values of a- & b-wave amplitudes in all groups.

Table 1: Mean values of a- and b- waves amplitudes (\pm SD) after 15 days in groups, G_{IA} and GII as compared to control.

ERG Parameters	Control	Group I_A	Group II
a-wave amplitude (mV)	4.82 \pm 0.77	1.68 \pm 1.56	2.96 \pm 0.52
b-wave amplitude (mV)	19.65 \pm 4.33	4.62 \pm 0.9	14.2 \pm 0.49
P_1		0.001*	0.001*
	a-wave		
	b-wave	0.000*	0.12
P_2			0.104
	a-wave		
	b-wave		0.000*
Mean values of a- and b- waves amplitudes (\pm SD) after 30 days in groups, control group, G_{IA} and GIII.			
a-wave amplitude (mV)	4.82 \pm 0.77	2.17 \pm 1.97	1.79 \pm 0.32
b-wave amplitude (mV)	19.65 \pm 4.33	9.80 \pm 1.89	11.01 \pm 2.94
P_1		0.12	0.000*
	a-wave		
	b-wave	0.000*	0.02
P_2			0.659
	a-wave		
	b-wave		0.416

Data expressed as mean \pm SD, n=6, μ V=microvolt, *significant difference.

P_1 value, t test, compares between a- & b- wave values of G_{IA} and GII to control group.

P_2 value, t test, compares between a- & b- wave values of G_{II} to G_{IA} .

Table 2: Mean \pm SD of lead concentration in blood and tissues (bone, liver and kidney) of rabbits administered lead acetate alone (G_{IA} and G_{IB}) or with vit. C (G_{II}) or treated with vit. C (G_{III}) as compared to control.

Parameters	Control	G_{IA}	G_{IB}	G_{II}	G_{III}
Blood lead Conc μ g/dL	10.3 \pm 0.56	173.4 \pm 13.56	121 \pm 7.45	125* \pm 2.67	44.86*** \pm 3.76
Bone lead conc. (μ g/100 g dry wt)	7.7 \pm 0.35	524.4*** \pm 44.56	434.543 \pm 58.34	353.144* \pm 31.45	434.11 \pm 33.56
Liver lead level (μ g/100 g wet wt)	13.3 \pm 1.56	114.25* \pm 9.43	77.39* \pm 6.04	34.72** \pm 1.45	11.89 \pm 4.67
Kidney lead level (μ g/100 g wet wt)	6.11 \pm 0.51	98.45 \pm 7.33	58.35* \pm 3.13	54.03** \pm 1.88	9.45 \pm 2.65

* $P < 0.01$, ** $P < 0.5$ *** $P < 0.001$ compare between each group and control

Table 3: Mean \pm SD of intrinsic viscosity, electrical conductivity, and magnetic susceptibility of hemoglobin as well as super oxide dismutase concentration in rabbits administered lead acetate alone (G_{IA} and G_{IB}) or combined with vit. C. (GII) or treated with vit. C. (GIII) as compared to control.

Parameters	Control	G_{IA}	G_{IB}	GII	GIII
Intrinsic viscosity (dL/gm)	0.0311 \pm 0.002	0.0391 \pm 0.002	0.0355 \pm 0.002	0.0371 \pm 0.002	0.0327 \pm 0.0076
Electrical conductivity (μ S/cm)	37.67 \pm 0.976	53.78 \pm 1.381	41.73 \pm 1.563	44.55 \pm 0.514	42.55 \pm 3.563
Magnetic susceptibility ($cm^3 \cdot mol^{-1}$)	- 0.897 \pm	- 0.612* \pm	-0.636 $\cdot 10^{-6} \pm$	- 0.798 $\cdot 10^{-6} \pm$	-0.782 \pm
	0.017 $\cdot 10^{-6}$	0.017 $\cdot 10^{-6}$	0.013 $\cdot 10^{-6}$	0.014 $\cdot 10^{-6}$	0.013 $\cdot 10^{-6}$
SOD (U/mL)	109.45 \pm 6.04	156.88 \pm 11.45	156.88 \pm 6.43	188.45 \pm 9.34	116.54 \pm 11.51

* $P < 0.01$, ** $P < 0.5$ *** $P < 0.001$ compare between each group and control

In this study the adverse effects of lead on retina have been demonstrated after 15 days of oral lead administration. The ERG alterations were correlated to histological changes. The amplitudes of a- and b-wave were significantly reduced below the normal values. The marked reduction of a-wave amplitude which reflects the photoreceptor dysfunction was documented by fragmentation and vacuolation of photoreceptor outer and inner segments. The damage of photoreceptors was extended to their nuclei in the outer nuclear layer which

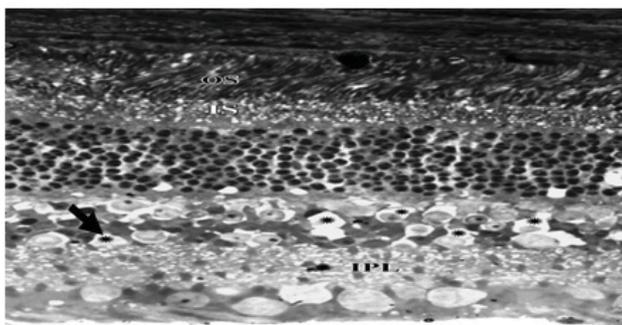


Fig. 4: The control albino rabbit retina is showing the pigment epithelium (PE), Photoreceptor layer (Ph.L), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL) and inner limiting membrane. (x 500).

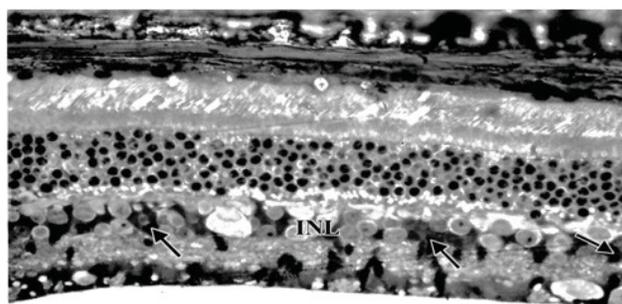


Fig. 5: The rabbit retina of GIA (which received lead acetate only) shows edema of all retinal layers. Photoreceptor layer shows disruption of the outer segment (OS) and vacuolation of inner segment (IS). Some cells of inner nuclear layer show a halo of clear cytoplasm (*). Inner plexiform layer (IPL) shows numerous glial cells (arrow). (x 500)

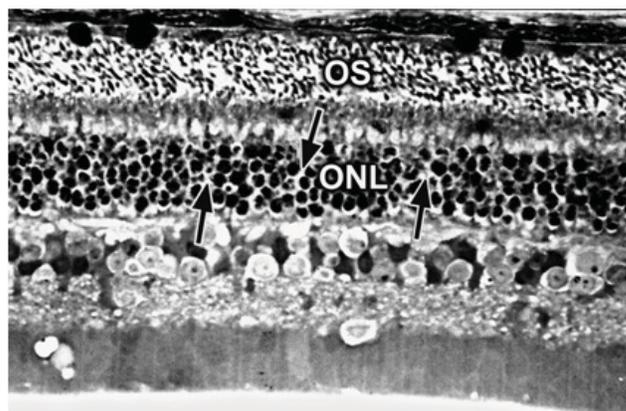


Fig. 6: The rabbit retina of GIB (recovery) is showing slight improvement of retina. Muller cells of inner nuclear layer (INL) appear dense (arrows). (x 500).

showed evident sign of pyknosis, karyorrhexis & karyolysis. Lead induced retinal toxicity has been previously proposed. There is evidence that in human lead exposure causes rod and bipolar apoptotic cell death (Erie *et al.*, 2005 and Fox *et al.*, 1997). (Rothenberg *et al.*, 2002) concluded that rods in the photoreceptor layer are sensitive target for lead as revealed by the defect in the ERG.

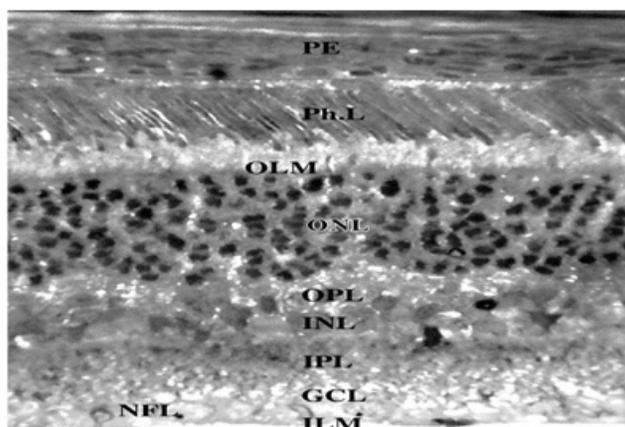


Fig. 7: Retina of rabbits of group (GII) that received lead and vitamin C showed edema of all retina layers. The outer segments of the photoreceptors appeared fragmented. Most nuclei of the outer nuclear layer were surrounded by a clear cytoplasm (arrows). However, the cells of the retina nuclear layer appeared swollen orth pale stained nuclei. (x 500).



Fig. 8: The retina of group (GIII) that received lead for 15 days and then vitamin c for other 15 days, showed sever damage to the photoreceptor layer (arrows) (x 500).

The present results showed that the cytoplasm of PE contained vacuoles and dense granules. (Bailey *et al.*, 2004) Ascribed those PE changes to lead induced oxidative stress. The latter disrupt the cell function and blood-retinal barrier integrity. Also the lead induced swelling of PE leads to degeneration of photoreceptors in rabbits (Erie *et al.*, 2005; Fox *et al.*, 1997 and Fox, 1998). These retinal changes did not recovered completely after stoppage of lead intake for another 15 days. The ERG a-wave showed some improvement but the b-wave amplitude was still significantly low. Histologically, Muller cells in the INL were dense. These results are in agreement with previous reports stated irreversible retinal histological changes (Ruan *et al.*, 1994) and electrophysiological components with lead intoxicification (El-Azazi *et al.*, 1987).

The toxic effects of lead could be related to its ability to substitute for divalent cations; calcium (see higher accumulation of lead in bone (Table 2) and zinc, in their binding sites in the cell and hence damaging multiple biological processes such as ionic conduction, metal transport, energy metabolism, apoptosis, cell adhesion, inter- and intracellular signaling, diverse enzymatic processes, protein maturation and genetic regulation (Garza and Vega, 2006). Also, lead has been suggested to generate reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage and alteration of antioxidant defense systems of cells represented by superoxide dismutase (SOD), catalase (Cat) and glutathione (GSH) (Ahmed and Siddiqui, 2007; Hsu and Guo, 2002). Another role was through the damage of phagocytic activity of Muller cells is by depleting these cells from their GSH content. This consequently affects the harmonical action of the other endogenous antioxidant as SOD and Cat.

Several metal chelators have been used to manage lead toxicity in the event of exposure, but none could be found suitable for reducing lead burden in chronic lead exposure (Osweiler, 1999). Moreover, these

chelators have a toxic potential in themselves (Gilman *et al.*, 1991) and often fail to remove lead from all body tissues (Bratton *et al.*, 1981 and Cory *et al.*, 1987). There is evidence that some nutrients, especially Vitamin C, exhibit some protective effects against intoxication with lead (El-Zayat *et al.*, 1996 and Houston and Johnson, 2000).

This study showed that administration of Vitamin C with lead exposed animals exerts an obvious ameliorating as well as treatment effects. The amelioratory effect of Vitamin C was more pronounced as ERG parameters were reversed toward normal values and retinal cytoarchitecture was much more ameliorated. This improvement might be related to its antioxidant efficacy that inhibits lipid peroxidation enhanced by lead. Vitamin C (ascorbic acid) is a low molecular mass antioxidant that scavenges the aqueous ROS by very rapid electron transfer that inhibits lipid peroxidation (Halliwell and Gutteridge, 1985). Administration of vitamin C significantly inhibited the lipid peroxidation levels of liver and brain, and increased the catalase (CAT) levels of kidney in lead-exposed rats (Patra *et al.*, 2001).

Lead is known to be toxic agent, and blood lead level is a convenient and direct indicator of such toxicity. However, lead could affect the rat erythrocyte membrane and decrease their motility (Terayama *et al.*, 1986). In addition, lead may induce oxidative stress in red blood cells (Gurer *et al.*, 1998). Moreover, erythrocytes can spread lead to different organs of the body (Sivaprasad *et al.*, 2003). Blood and tissues lead levels exerted a dramatic reduction after the administration of Vitamin C (GIII) which is verifying the chelating potency to lead ions. A group that left to self recovery (GIB) showed a significant lead reduction but less than those treated with Vitamin C (GIII). Rabbits administered lead with Vitamin C (GII) provided better recoveries against lead and this might be attributed to early chelation of lead ions upon the first stages of poisoning.

Lead-induced oxidative stress in blood and other soft tissues has been postulated to be one of the possible mechanisms of lead-induced toxic effects (Pande *et al.*, 2001). Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation (Upasana and Balaraman, 2001) and altered the antioxidant defense system (Adanaylo, 1999). Several antioxidant enzymes and molecules have been used to evaluate lead-induced oxidative damage in animal and human studies. Modifications in SOD activity are the most frequently used markers in tissues or in blood. Based on the observation that free radical was generated during the pathogenesis processes induced by lead exposure, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy (Flora *et al.*, 2003). Specifically, ascorbic acid, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress (Ramanathan *et al.*, 2002 and Patra *et al.*, 2001). Besides, additional protective effect of vitamin C on cell apoptosis was revealed in a recent study (Gruss and Fabian, 2002).

An important finding from our work is the combination between the oxidative stress resulted from lead poisoning and its effects on the biophysical parameters of hemoglobin as a try to evaluate the mode of action by which lead can alter the efficiency of the retina cells. Results had shown a dramatic decrease of lead ions in both lead and tissue except that in bone. Until now no feedback mechanism is listed to lead removal from bone. Some investigators had indicated that lead exposure increased the production of ROS in the liver (Pande *et al.*, 2001 and Pande and Flora, 2002). This mechanism can be insured by the elevated activity of SOD in animals that received lead. After Vitamin C treatment application this activity fall, to be approximately normal, this may be explained as the formation of ROS was nearly stopped after lead ions had been removed from blood. Another finding is the high alterations in the biophysical parameters of hemoglobin regarding its shape and size in level of one molecule. Changes measured in viscosity, electrical conductivity and magnetic stability of hemoglobin were reversed with the treatment application. These changes may play a complementary role in the oxidative stress of lead poisoning, as the hemoglobin molecule suffers from shape and size as well as magnetic susceptibility to the extend it may not able to carry out its normal function. This function disability may result in shortage in the molecular oxygen supply to tissues that will magnify the oxidative stress of lead. Another beneficial role of ascorbic acid supplementation in lead- exposed rats was associated with serum biochemical alterations in the haemopoietic system and drug metabolizing enzymes. Intraperitoneal administration of lead in rats produced a significant inhibition of heme synthesis in blood and liver, and reduced drug metabolism in liver. Vitamin C supplementation in lead-exposed animals significantly reduced blood, liver, and renal lead levels. This result indicated a significant protective action of vitamin C against toxic effects of lead on heme synthesis and drug metabolism (Vij *et al.*, 1998). A study found the combination of vitamin C and thiamine was effective in reducing lead levels in blood, liver, and kidney. (Anderson *et al.*, 1994) showed that there were small protective effects of Vitamin C at low doses and exacerbating effects at high doses.

In conclusion, the combined administration of lead-exposed rabbits with 40 mg Vitamin C / Kg body weight did not confer significant protection against lead toxicity but showed marked improvement of the ERG parameters, histopathological, biochemical and biophysical findings. Better results were obtained when treatment started after complete stoppage of intoxication process. (Laila *et al.*, 2005) studied the protective role of vitamin C versus vitamin E on retina toxicity induced by lead in rats. They reported that vitamin E can restore the retina histological pattern better than vitamin C. they attributed this protection to the fact that vitamin E lies within the lipid layer of the cellular, organellar and nuclear membrane, thus restoring their structure and hence their function.

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