PROPHYLACTIC EFFECT OF *ANGELICA ARCHANGELICA* AGAINST ACUTE LEAD TOXICITY IN ALBINO RABBITS

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Abstract. The aim of the present study was to investigate the prophylactic action of *Angelica archangelica* against lead toxicity. The adverse effects of lead on the blood and retina 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. Administration of *Angelica archangelica* in combination with lead exerts obvious prophylactic as well as therapeutical effects. The prophylactic effect of *Angelica* was more pronounced as ERG parameters were reversed to close the normal values and retinal cytoarchitecture was much ameliorated. Oral administration of lead caused an elevation of blood, bone, liver and kidney lead level as compared with control group. The biophysical parameters of blood (magnetic susceptibility, electrical conductivity, viscosity, and super oxide dismutase activity) were estimated. The results showed that the administration of lead induced significant alterations in these parameters. Upon the administration of *Angelica archangelica* in combination with lead recovered such alterations to near the control levels. The ability of *Angelica* to reduce lead toxicity may relate to its antioxidant and chelation action. Supplementation of diets with *Angelica* may be recommended to improve the body burden of lead and hence to protect the organ function against lead toxicity.

Key words: Lead toxicity, *Angelica archangelica*, ERG, histopathology, hemoglobin biophysics, prophylactic effect.

INTRODUCTION

Lead is an industrial pollutant that has been detected in almost all phases of environmental and biological systems. The quantity of lead used in the 20th century far exceeds the total consumed in all previous eras. This heavy use has caused local and global contamination of air, dust, and soil.

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Lead is known to induce a broad range of physiological, biochemical, and behavioral dysfunctions in laboratory animals and humans [15, 30], including central and peripheral nervous systems [6], haematopoietic system [8], cardiovascular system [20], kidneys [19], liver [34], and reproductive systems [21, 27]. However, the nervous system damage is considered the most serious. Neurological and visual alterations (including retina) have been reported with low lead concentration particularly in the developing nervous system. 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 [35].

A great part of damage induced by lead in cellular physiology is caused by its ability to substitute for diverse polyvalent cations (calcium, zinc, and magnesium) in their binding sites [14]. Oxidative stress associated with the presence of lead in mammalian tissues and organs (predominantly blood, liver, kidneys and brain) appears to be one of the possible molecular mechanisms for lead toxicity that has been postulated by researchers. Therefore, lead intoxication is currently treated with ethylene diamine tetracetic acid (EDTA) for its calcium binding activity and antioxidant effects [17].

The clinical importance of the herbal drugs has recently received considerable attention. Their role, though, has mainly been limited to act as an antioxidant and to provide better recovery in the altered biochemical variables. None of them has been found so far to act as a lead chelator.

Angelica is one of the famous and oldest plants that have been used therapeutically for centuries. It is a natural antioxidant. *Angelica archangelica* (European *Angelica*) is a member of a widely cultivated *Angelica* herb. It has been used in traditional medicine as a remedy for various disorders such as: headaches, backaches, asthma, skin disorders and digestive disorders [32]. Being composed of many chemical components including essential oils, organic acids, steroids, coumarines and flavonoids several pharmacological actions of the plant root have been studied. *Angelica archangelica* root was reported to have analgesic, antiseptic, anti-inflammatory, antifungal, antispasmodic, and anti-cancer cell proliferative properties as well as calcium channel blocking and calcium binding activity. The main constituents of essential oils of *Angelica archangelica* root are pynene, limonene and coumarin osthol that were recently stated to possess antioxidant effects [25] and macrocyclic lactones that exhibit calcium binding activities. Based on these constituents of *Angelica archangelica*, we conjectured that if *Angelica* can confer protection and provide therapeutic action against lead toxicity, much of the side effects of lead detoxification can clearly be avoided.

The aim of the present work is to follow the adverse effects of lead in the retina, bone, liver and kidneys and to estimate the supposed chelating activity of *Angelica* considering the biophysical effects of lead in blood. The findings could be useful to understand lead toxicity and useful protection.

MATERIAL AND METHODS

CHEMICALS AND HERBS

Lead acetate was purchased from Merk (Darmstadt, Germany). Lignocaine hydrochloride and ketamine hydrochloride were purchased from Amoun (Pharmaceutical Company, Egypt). Glutaraldehyde was obtained from Boehringer-Ingelheim, Germany .The roots of *Angelica archangelica* (whole root, first grade) were provided and identified by Mr. Haraz (Famous and Oldest Egyptian Herbs and folk medicine manufactures and suppliers, Cairo, Egypt) in March 2007. The samples of *Angelica* were harvested from Syria in August 2006. All chemicals were used without further purification.

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 with 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 which received distilled water for 30 days. Group **I** consists of 6 rabbits which received oral lead acetate (in water with concentration of 12.5 mg/kg body weight) for 15 days, 3 rabbits were subjected to Electro Retino Graphic (ERG) and light microscopy examination (group $\mathbf{I_A}$), while the other 3 rabbits left free untreated for another 15 days to study the possible recovery (group $\mathbf{I_B}$). Group **II** consists of 3 rabbits which received two times per day both of *Angelica archangelica* roots powder water extraction with concentration of 0.11 g/kg body weight and lead acetate for 15 days. Group **III** consists of 3 rabbits which received lead acetate for 15 days, and then were treated with *Angelica archangelica* roots powder water extraction 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 (5 mg/kg) and ketamine hydrochloride (50 mg/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 no 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 was 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 [4].

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 method [2]. The force excreted 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:

$$K_{\mathrm{S}} = \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_{\mathrm{W}} + [1 - \frac{\delta S}{S}\frac{W}{\delta W}\frac{\rho}{d}K_{\mathrm{a}}] \tag{1}$$

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 by:

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

DETERMINATION OF HEMOGLOBIN ELECTRICAL CONDUCTIVITY

Conductivity of hemoglobin solution was determined using a conductivity meter type digimeter L21/L21 C aqualytic 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 – 200 μS/cm). The conductivity meter was calibrated before measurements using a standard solution.

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 [31].

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 [37].

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:

$$\frac{\eta}{\eta_0} = \frac{\rho t}{\rho_0 t_0} \tag{4}$$

where η, ρ and t are the viscosity, density and the time of flow of hemoglobin solution respectively. η_o, ρ_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 (η_{sp}) is given. The method is repeated for several concentrations and a relation between η_{sp} and C is plotted; the result will be a straight line, its extrapolation giving the intrinsic viscosity.

DETERMINATION OF SUPER OXIDE DISMUTASE ACTIVITY

Determination of super oxide dismutase (SOD) activity was carried out by a RANDOX kit package [24]**.**

STATISTICAL ANALYSIS

Amplitudes of a- and b-waves of ERG were expressed as the mean wave amplitude ± 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

ELECTROPHYSIOLOGICAL RESULTS

ERG results are shown in Tables 1 and 2 and Figures 1, 2, and 3. Before the study, ERG recordings showed no significant differences between the control group and the other groups ($P > 0.05$, ANOVA). In group I_A, Oral administration of lead for 15 days results in significant reduction in a- and b-wave amplitudes in comparison with control group (Table 1, Fig. 1).

After ceasing the lead administration for another 15 days (group $\mathbf{I_B}$), the value of a-wave amplitude was improved but still lower than control eyes, while the b-wave amplitude was significantly decreased in comparison to control group (Table 2, Fig. 2).

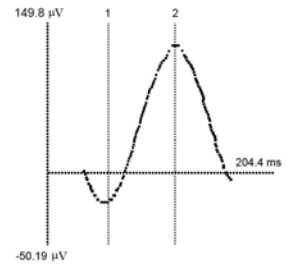
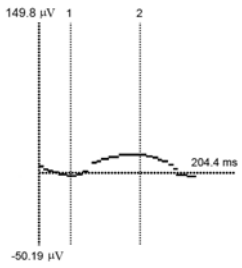
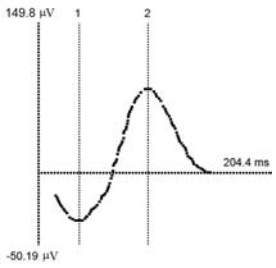
Groups	Control	G I_A	G II
			
a Wave	**3.92**	**0.10**	**6.00**
b Wave	**19.60**	**3.14**	**15.07**

Fig. 1. ERG recordings of selected rabbit eyes in group II, I_A and control group. ERG combined response demonstrated marked reduction of a- and b-wave amplitudes in group I_A. Both a- and b-wave amplitudes were within normal in group II.

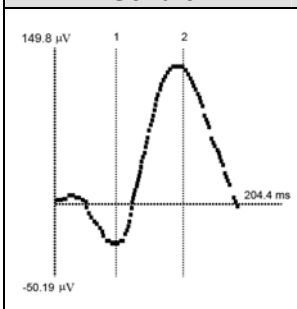
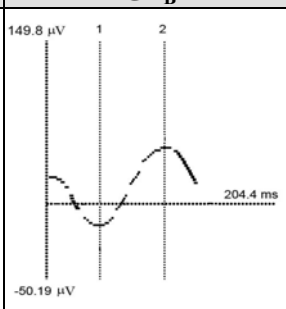
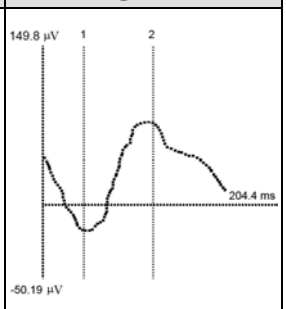
Groups	Control	G I_B	G III
			
a-wave	**5.00**	**2.07**	**2.50**
b-wave	**22.00**	**10.58**	**12.59**

Fig. 2. ERG recordings of selected rabbit eyes in group **III**, $\mathbf{I_B}$ and control group. ERG combined response demonstrated improved a- and b-wave amplitudes in group **III**. The b-wave amplitudes were still decreased in group $\mathbf{I_B}$.

Administration of *Angelica* with lead (group **II**) maintained ERG parameters near to normal values. Statistical analysis showed no significant difference in a-and b-wave amplitudes between (group **II**) and control group, while they were significantly high in comparison with group $\mathbf{I_A}$ (Table 1, Fig.1). Also in group **III** (eyes treated with *Angelica*) both a- and b-wave amplitudes were significantly improved. In comparison to control group no significant difference was found in a- or b-wave amplitude while there was a significant difference in b-wave amplitude between group $\mathbf{I_B}$ and group **III** (Table 2, Fig. 2).

Table 1

Mean values of a- and b-waves amplitudes (±SD) after 15 days in group (control rabbits), group **I_A** (received lead) and group **II** (received lead with *Angelica*)

ERG parameters		**Control**	**Group I_A**	**Group II**
a-wave amplitude (μV)		4.12 ± 0.77	1.44 ±1.45	4.19 ± 0.25
b-wave amplitude (μV)		16.93 ± 2.20	5.67 ± 1.36	15.57 ± 1.21
P_1	a-wave		0.002 *	0.827
	b-wave		0.000 *	0.216
P_2	a-wave			0.001*
	b-wave			0.000 *

Data expressed as mean ± SD, *n* = 6, *significant difference.
P1 value, t test, compares between a- and b-wave values of group **I_A** and **II** to control group.
P2 value, t test, compares between a- and b-wave values of group **II** to group **I_A**.

Table 2

Mean values of a- and b-waves amplitudes (±SD) after 30 days in group (control rabbits), group **I_B** (received lead) and group **III** (treated with *Angelica*)

ERG parameters		**Control**	**Group I_B**	**Group III**
a-wave amplitude (μV)		4.23 ± 0.74	2.66 ± 2.29	3.58 ± 1.95
b-wave amplitude (μV)		17.24 ± 3.16	9.80 ± 1.88	14.5 ± 2.96
P_1	a-wave		0.141	0.459
	b-wave		0.001*	0.153
P_2	a-wave			0.475
	b-wave			0.008*

Data expressed as mean ± SD, *n* = 6, *significant difference.
P1value, t test, compares between a- and b-wave values of group **I_B** and **III** to control group.
P2 value, t test, compares between a- and b-wave values of group **III** to group **I_B**.

Both a-wave and b-wave ERG amplitudes for all groups were considered in Fig. 3.

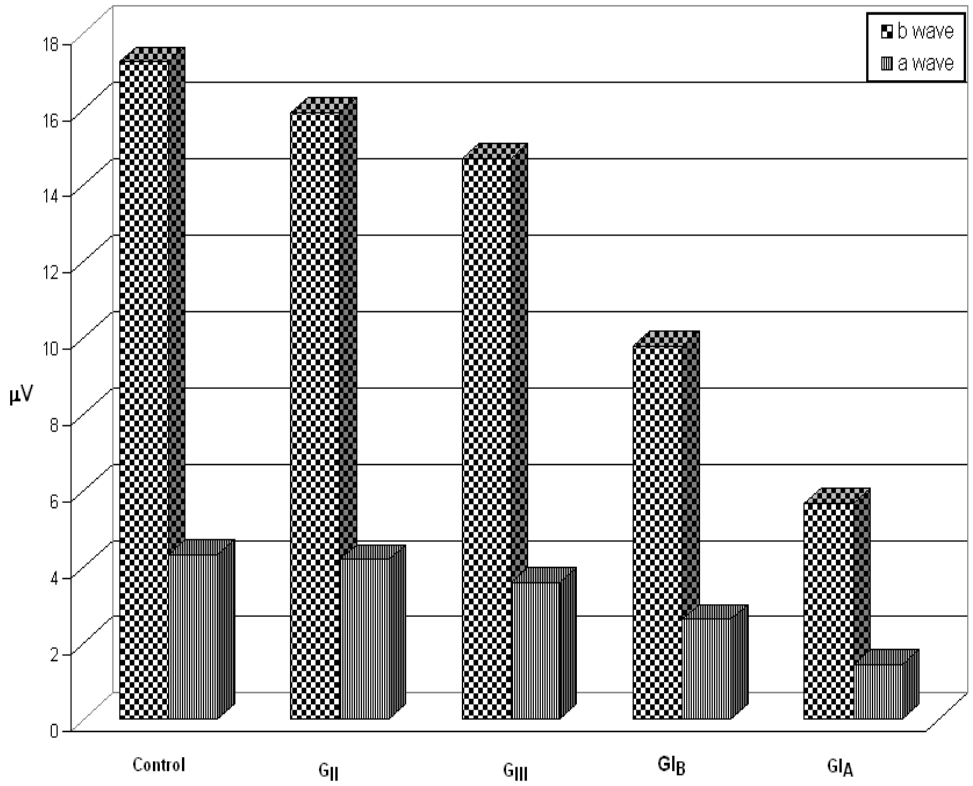


Fig. 3. The mean values of a- and b-wave amplitudes in all groups.

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 group $\mathbf{I_A}$, which received lead acetate only, revealed edema of all layers of the retina and several distinct alterations (Fig. 5). The cytoplasm of the pigment epithelium 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, and karyolysis.

The cells of the inner nuclear layer (INL) and ganglion cell layer (GC) appeared swollen. Some cells of INL showed hallo of clear cytoplasm. Inner plexiform layer contained numerous glial cells (Fig. 5).

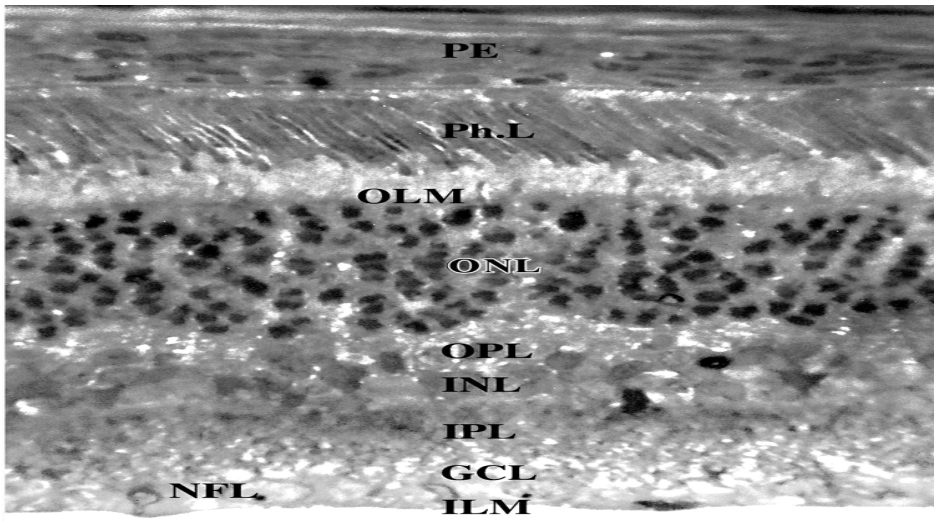


Fig. 4. The control albino rabbit retina is showing the pigment epithelium (PE), photoreceptor layer (Ph.L), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL)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

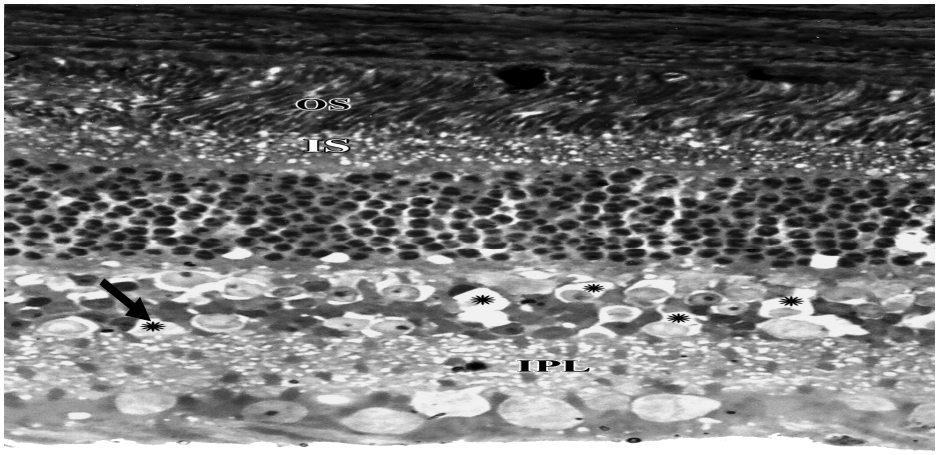


Fig. 5. The rabbit retina of group I_A (which received lead acetate only) shows edema of all retinal layers. Photoreceptor layer shows disrupter of the outer segment (OS) and vacuolation of inner segment (IS). Some cells of inner nuclear layer show a hallo of clear cytoplasm (*). Inner plexiform layer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

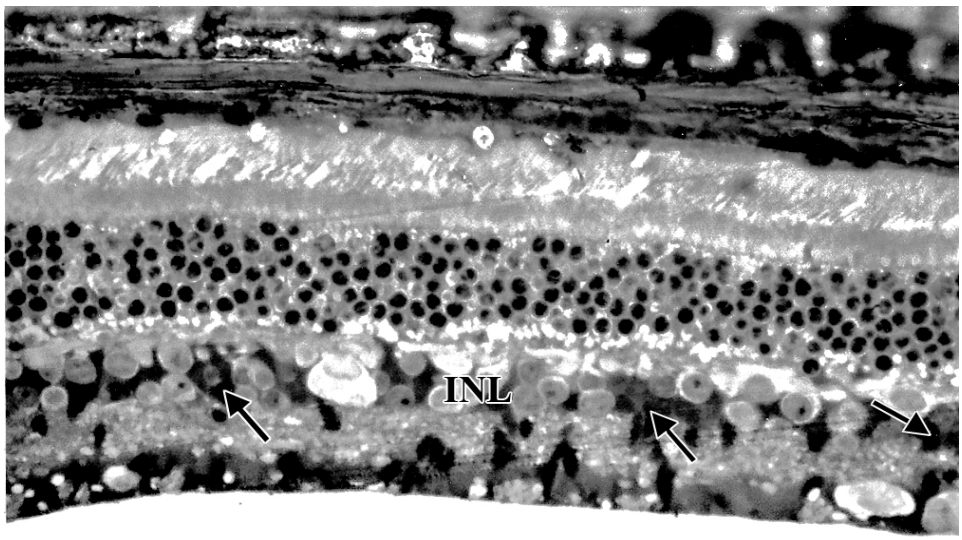


Fig. 6. The rabbit retina of group IB (recovery) is showing a slight improvement of retina. Muller cells of inner nuclear layer (INL) appear dense (arrow) (× 500).

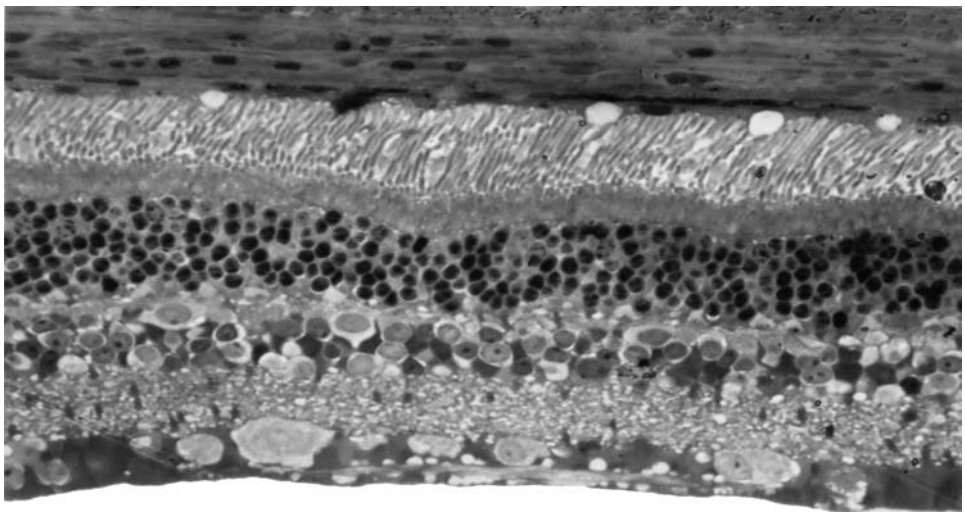


Fig. 7. The rabbit retina of group II (protection) is showing a normal histological picture of all layers (× 500).

The rabbits in group I_B revealed slight improvement of photoreceptor layer; in addition, edema of the retina in animals intoxicated with lead was subsided. However, Muller cells in the INL appeared dense (Fig. 6).

Histological examination of the retina of rabbits in group II, which received lead acetate with *Angelica*, showed a normal histological picture (Fig. 7) while examination of rabbit retina of group III, which received lead acetate then treated with *Angelica*, showed improvement in retinal layers (Fig. 8).

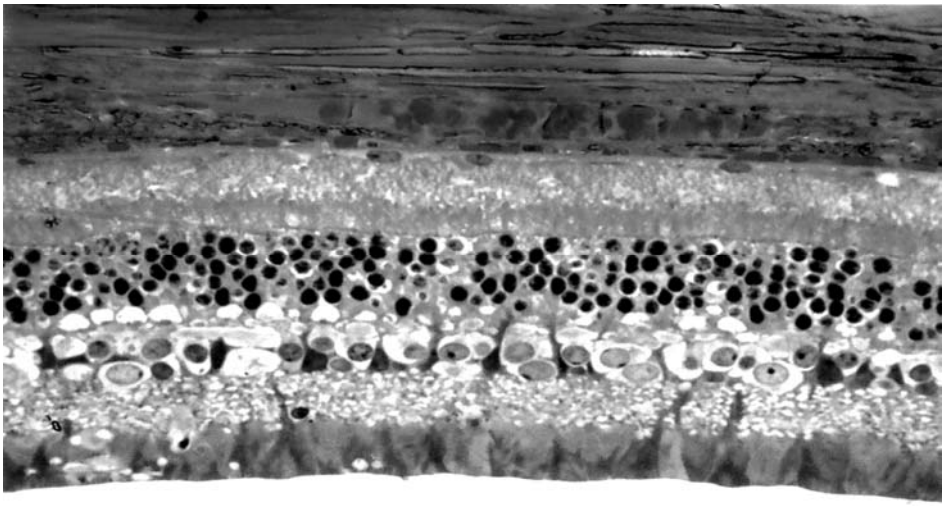


Fig. 8. The rabbit retina of group III is showing an improvement of retinal layers (× 500).

Table 3 represents blood, bone, liver and kidney lead level of rabbits under normal conditions, after oral administration of lead acetate alone (GI_A and GI_B) or with *Angelica archangelica* (G_{II}) or treated with *Angelica archangelica* (G_{III}). Administration of lead acetate caused an increase in blood lead level and the other tissues while bone underwent a significant increase more than the others. As indicated in Table 3, animals which received lead with *Angelica* (G_{II}) lowered the blood and tissues lead level to significant values when compared to control levels.

Table 4 represents the biophysical parameters of blood for rabbits under normal conditions, after oral administration of lead acetate alone (GI_A and GI_B) or with *Angelica archangelica* (G_{II}) or treated with *Angelica archangelica* (G_{III}). As depicted from Table 4, Animals which received lead with *Angelica* (G_{II}) registered an improvement in the intrinsic viscosity as compared to control value. A significant increase was recorded in electrical conductivity for the group administered lead acetate alone without treatment (G_{IA}). Magnetic susceptibility of rabbits administered lead with *angelica* extraction (G_{II}) exerted a high diamagnetic negative value as compared to either lead intoxicated rabbits or treated groups. The SOD activity value in G_{II} group recorded a value close to that of control.

Table 3

Mean ± SD of lead concentration in blood and tissues (bone, liver and kidney) of rabbits administered with lead acetate alone (GI_A and GI_B) or with *Angelica* (G_{II}) or treated with *Angelica* (G_{III}) as compared to control

	Control	**G_{IA}**	**G_{IB}**	**G_{II}**	**G_{III}**
Blood lead conc (µg/dL)	9.45 ± 0.56	156.45 ± 13.56	87.45 ± 7.45	32.66 ± 2.67	43.45 ± 3.76
Bone lead conc. (µg/100 g dry wt)	4.74 ± 0.35	524.4 ± 44.56	434.543 ± 38.34	358.178 ± 31.45	434.11 ± 33.56
Liver lead level (µg/100 g wet wt)	13.45 ± 1.56	114.25 ± 9.43	77.39 ± 6.04	11.89 ± 1.45	54.92 ± 4.67
Kidney lead level (µg/100 g wet wt)	6.75 ± 0.51	98.45 ± 7.33	58.35 ± 3.13	9.45 ± 1.88	34.23 ± 2.65

Table 4

Mean ± SD of intrinsic viscosity, electrical conductivity, and magnetic susceptibility of hemoglobin as well as super oxide dismutase concentration in rabbits administered with lead acetate alone (GI_A and GI_B) or with *Angelica* (G_{II}) or treated with *Angelica* (G_{III}) as compared to control

	Control	**G_{IA}**	**G_{IB}**	**G_{II}**	**G_{III}**
Intrinsic viscosity (dL/gm)	0.0311 ± 0.002	0.0391 ± 0.002	0.0357 ± 0.002	0.0327 ± 0.002	0.0355 ± 0.0076
Electrical conductivity (µS/cm)	37.67 ± 0.976	53.78 ± 1.381	43.51 ± 3.45	42.55 ± 0.514	41.73 ± 3.563
Magnetic susceptibility ($cm^3.mol^{-1}$)	– 0.897 ± 0.017×10^{-6}	– 0.612 ± 0.017×10^{-6}	-0.754×10^{-6} ± 0.013×10^{-6}	-0.782×10^{-6} ± 0.014×10^{-6}	-0.636×10^{-6} ± 0.013×10^{-6}
SOD (U/mL)	109.45 ± 6.04	156.88 ± 11.45	146.11 ± 6.43	116.54 ± 9.34	136.88 ± 11.51

DISCUSSION

Lead exposure continues to be a major public health problem, particularly in the third world [7] as a result of research on the toxic effects of lead continues and the last decade has been particularly fruitful in providing new information on the manifold influences of this metal [22]. Neurotoxicity from lead exposure is of concern especially because lead at even very low concentrations can have profoundly detrimental neurological effects [5].

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 b-wave amplitude which is highly dependent on bipolar cells and results from current flow along Muller cells was reduced and corresponding to the histological picture of the INL which showed evident signs of pyknosis, karyorrhexis and karyolysis. Lead induced retinal toxicity has been previously proposed. There is evidence that in human lead exposure causes rod and bipolar apoptotic cell death [10, 11]. Rothenberg *et al.* [28] concluded that rods in the photoreceptor layer are a sensitive target for lead as revealed by the defect in the ERG.

The present results showed that the cytoplasm of PE contained vacuoles and dense granules. Bailey *et al*. [3] 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 [10, 11, 12].

These retinal changes did not recover 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, which are in agreement with previous reports, stated irreversible retinal histological changes [29] and electrophysiological components with lead intoxication [9, 33].

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 3) 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 [13]. 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 SOD, Cat and GSH [1, 18]. Another role was through the damage of phagocytic activity of Muller cells by depleting these cells from their GSH content. This consequently affects the harmonical action of the other endogenous antioxidants as SOD and Cat.

This study showed that administration of *Angelica archangelica* with lead exerts obvious prophylactic as well as treatment effects. The prophylactic effect of *Angelica* was more pronounced as ERG parameters were reversed to normal values and retinal cytoarchitecture was much more ameliorated. This improvement might be related to more than one component included in *Angelica* root essential oils; the macrocyclic lactones that exhibit calcium binding activities and might resemble the effect of EDTA on lead intoxification, and α-pynene, limonene, and the coumarin osthol that possess antioxidant properties [23, 25, 26].

Lead is known to be a 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 [36]. In addition, lead may induce oxidative

stress in red blood cells [16]. Moreover, erythrocytes can spread lead to different organs of the body [35].

Blood and tissues lead levels exerted a dramatic reduction after the application of water extraction of *Angelica* roots powder verifying the chelation potency of this herb to lead ions. A group that left to self recovery (G_{IB}) showed a significant lead reduction but less than those treated with the herb (G_{III}). Rabbits administered lead with *Angelica* (G_{II}) provided better recoveries against lead and this could be attributed to early chelation of lead ions upon the first stages of poisoning. These results are in accordance with the results of the ERG.

The late effect of lead ions on blood appears as the lower activity of the super oxide dismutase enzyme hence it is a direct indicator of the rate by which free radicals are formed. In other words, the decrease in free radicals concentration may coincide with higher antioxidant activity of the chelator itself as it may contain EDTA like substances that may play a role in the scavenging of the reactive oxygen species. The ability of *Angelica* to reduce lead toxicity may rely on its antioxidant/chelating action.

An important aspect in the study of biophysical parameters of the hemoglobin shape, size and magnetic properties is to understand lead toxicity and useful protection. Magnetic properties showed a higher steadiness of the hemoglobin molecule in the group that received *Angelica* in combination with the poisoning lead, as the high negative value of the magnetic susceptibility revealed that the hemoglobin is still in the diamagnetic region in which the ferro-ferric transition is ideal to maintain normal behavior of hemoglobin and to carry out normal functions of blood. The increase in the electrical conductivity for hemoglobin as compared to control could be attributed to the increased free radicals formation rate, reactive oxygen species, and peroxide radicals which result from lead toxicity, therefore there is an increase in the surface charge density of hemoglobin macromolecule. As depicted from Table 4, higher electrical conductivity was recoded upon lead administration to rabbits. Highly organized activity of hemoglobin molecules is obviously established with the herbal treatment administration.

No previous investigation has examined the effect of *Angelica* on lead retinal toxicity. We investigated the beneficial effects of *Angelica archangelica* in protecting retinal rabbits against lead induced oxidative damage suggesting the removal of lead from the target tissue. It was found that *Angelica archangelica* inhibits the malondialdehyde formation in mouse liver homogenates both *in vitro* and *in vivo*. *Angelica archangelica* is an effective cytoprotective agent, possibly through inhibition of the production of oxygen free radicals that causes lipid peroxidation, and hence indirectly protects the liver from oxidative stress [38].

In conclusion, lead could have toxic effects which manifest in visual and hematological changes. Supplementation of diet with *Angelica* may be recommended to improve the body burden of lead and hence to protect the organ

function against lead toxicity. The exact mechanisms of *Angelica*-induced improvement are unclear and may be influenced by many other components of *Angelica*. This provides a strong impetus for further investigation.

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