BIOPHYSICAL AND BIOCHEMICAL CHANGES IN THE BLOOD OF RATS EXPOSED TO LEAD TOXICITY

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Abstract. The present work is devoted to study the lead (Pb) toxicity in experimental Wistar male rats exposed to 2% lead acetate in drinking water and hence to evaluate the risk on human workers who were environmentally and occupationally exposed to similar toxicity. A total of thirty two Wistar male rats were equally divided into four groups, A, B, C, and D. Group A served as control group. Group B was exposed to 2% lead acetate in drinking water for one month. Groups C and D were exposed to the same condition as Group B for two months and three months respectively. Dielectric dispersion of hemoglobin (Hb) at frequency range of $20–3\times10^6$ Hz, hemoglobin absorption spectra, plasma alanine transaminase (ALT), aspirate transaminase (AST), alkaline phosphatase (ALP), total protein, and cholesterol levels were carried out for all groups. The results indicated that exposure of animals to lead results in an increase in ALT, AST, ALP, and cholesterol levels in the plasma of one, two and three months respectively indicating some damage in the liver cell membrane. On the other hand, plasma total protein decreases significantly in the rats treated with lead. The dielectric results indicated that the studied hemoglobin of the lead treated groups has a dielectric dispersion in the frequency range used. The increase in the electrical conductivity and relaxation time for hemoglobin as compared to control could be attributed to the increased free radicals, reactive oxygen species, and peroxide radicals which results from lead toxicity, therefore there is an increase in the surface charge density of hemoglobin macromolecule. It was concluded that oral exposure of lead causes alterations in liver functions and biophysical parameters of hemoglobin.

Key words: Lead toxicity, dielectric dispersion, electrical conductivity, hemoglobin, ALT, AST, ALP.

INTRODUCTION

Lead is being an ubiquitous environmental contaminant due to its significant role in modern industry [38]. However, both occupational and environmental exposures remain a serious problem in many developing and industrializing countries [47]. It has many undesired effects, including neurological [29, 40],

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behavioural [8, 30], immunological [4, 5, 11, 34], renal [27, 32, 46], hepatic [32], and especially haematological dysfunctions [31, 39]. 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 [39].

Recent studies have shown that lead toxicity facilitates conversion of Hb into met-Hb. During Hb oxidation in the presence of lead, H_2O_2 is generated, which may induce lipid peroxidation in the erythrocyte cell membrane [46]. As a result, lead might induce generation of ROS by interacting with oxy-Hb, leading to peroxidative damage of erythrocyte membranes [36]. Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids [6], a deleterious process solely carried out by free radicals [20]. Many studies have investigated a possible relationship between lipid peroxidation (LPO) and cellular damage in hepatic tissues under various pathological conditions [7]. Lewis *et al*. [26] have suggested that peroxide formation may lead to oxidative destruction of thiol groups of amino acids and proteins. Lead can cause derangement of several hepatic biochemical pathways and energy metabolism [42]. In particular lead causes transient, but marked hypercalcemia, which may contribute to hepatotoxicity [43].

Dielectric properties of various biological materials have been previously investigated to get attractive information about their structural change under any internal or external effects [16, 22, 33].

Therefore, in this study, the effects of lead exposure on some biochemical and biophysical parameters of rat blood were investigated to evaluate the risk on persons who environmentally and occupationally exposed to similar toxicity.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Thirty two male Wister rats were used in this investigation. The average weight of the rats was 150±4.6 g. The animals were maintained under standard laboratory conditions (12 h light, temperature 23 °C). They were fed dry ration *ad libitum*. The animals were divided equally into four groups.

ADMINISTRATION OF LEAD ACETATE

The animals were treated daily for three months as follows:

Group A: served as control and was given tap water.
Group B: was treated with 2% lead acetate in drinking water for one month [3].
Group C: was treated with 2% lead acetate in drinking water for two months.
Group D: was treated with 2% lead acetate in drinking water for three months.
Lead acetate was obtained from Merk (Darmstadt, Germany).

At the end of the exposure time, each animal of all groups was anaesthetized with ether, and then blood samples were collected by heparinated capillary tubes from eye vein in heparin containing tubes. The plasma and hemoglobin solution were prepared according to method of [45]. The following measurements were performed:

BIOCHEMICAL ASSESSMENTS

Alanine aminotransferase (ALT), aspirate aminotransferase (AST) activites [35], alkaline phosphatase (ALP) [24], total protein [18], and cholesterol [1] were determined colorimetrically using kits from Bio Merieux, France.

HEMOGLOBIN ABSORPTION SPECTRUM

Hemoglobin absorption spectra of control and lead treated rats were measured in the wavelength range 200–700 nm [9], using a UV/Visible spectrophotometer type (Helios Alpha) 9423 NC.

DIELECTRIC MEASUREMENTS

Dielectric measurements were made in the frequency range from 20 Hz to 3 MHz using a WAYNE KERR precision component analyzer, model 6440 (UK), connected with a conductivity cell type 19250-60 manufactured by Cole Palmer Co. Hemoglobin solutions were placed in a homemade dielectric cell which contains two squared platinum black electrodes each having an area of 1 cm^2 with an inner electrode distance of 1 cm. The measurements were performed at constant temperature (20 ± 0.5 °C).

For a dielectric material between two parallel plates capacitor, the measured values of capacitance C and resistance R were used to calculate the real, ε', and imaginary, ε'', parts of the complex permittivity, ε^*; using the following equations:

$$\varepsilon' = \varepsilon_\circ C K \tag{1}$$

where $K = 1\ cm^{-1}$, is cell constant that depends on the cell dimension

$$\sigma = \frac{K}{R} \tag{2}$$

where σ is the conductivity in $\Omega^{-1}\,m^{-1}$,

$$\tau = \frac{1}{2\pi f_c} \tag{3}$$

where τ is the relaxation time in ms and f_c is the critical frequency in Hz, corresponding to the mid point of the dispersion curve.

STATISTICAL ANALYSIS

Each value is expressed as mean and standard error (SE). One way analysis of variance (ANOVA) was used to compare each variable in the different studied groups. For all statistical comparisons a value of $p < 0.01$ was considered significant.

RESULTS

The effect of lead toxicity on plasma total protein, cholesterol and liver functions which includes aspirate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) for treatment periods of one, two, and three months were measured and summarized in Table 1. It is clear from the table that all treatment periods showed a significant ($p < 0.01$) decrease on the plasma total protein and a significant ($p < 0.01$) increase on the other parameters (ALT, AST, ALP, cholesterol) as compared to control. These changes are time dependent.

Figure 1 shows the absorption spectra of hemoglobin (Hb) extracted from treated and untreated animals. The Hb spectra indicate the appearance of the well-known hemoglobin characteristic bands at 220, 280, 340, 410, 540, and 578 nm. These bands correspond to aliphatic amino acids, aromatic amino acids, globin-heme interaction, Soret band, nitrogen ion bonds in porphyrine rings and heme-heme interaction band, respectively. The absorption intensity decreased as the treatment period increased as compared to control. Moreover, no change in the peak position was noticed.

Figure 2 shows the electrical conductivity σ (a) and dielectric constant ε' (b) as a function of the frequency applied in the range of 20 Hz to 3 MHz for Hb of control (■) and treated rats for three months (●). The results indicate a strong dielectric dispersion for the studied samples. This behavior was identified as

anomalous frequency dispersion and it was found in different biological materials [12, 13, 16, 19, 41]. It is clear from the figure that there is a relatively significant increase in both values of ε' and σ for the treated group when compared with the control. The dielectric increment Δε' and relaxation time τ for the Hb molecules of the studied groups were calculated and given in Table 2.

Table 1

Effect of lead on plasma total protein, AST, ALT, ALP, and cholesterol

Parameter	Total proteins (g/L)		AST (μM/L)		ALT (μM/L)		ALP (μM/L)		Cholesterol (mg/100ml)	
Time (month)	control	treated	control	treated	control	treated	control	treated	control	treated
1	78.14 ±3.50	62.32* ±3.00	59.71 ±1.09	90.50* ± 3.00	24.85 ±0.60	32.19* ± 0.35	199.30 ±5.42	210.00 ±8.66	70.36 ± 2.14	80.05 ±6.83
2	81.53 ±5.26	55.62* ± 1.70	50.22 ±0.98	115.14* ±7.80	28..06 ±1.30	40.26* ±1.09	210.00 ±6.09	280.63* ±9.14	73.55 ± 4.10	120.35* ± 6.30
3	80.43 ±1.85	48.35* ±2.31	60.14 ±1.50	145.19* ±9.36	27.35 ±0.67	65.98* ± 2.03	202.50± 10.36	300.46* ±11.01	79.38 ± 3.59	140.77* ± 8.50

Values represent mean ± SE, n = 8.
*: significant difference from control.

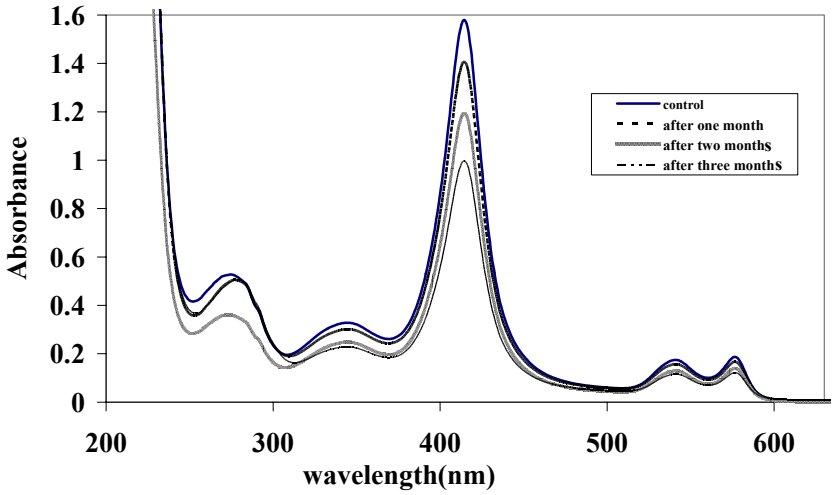
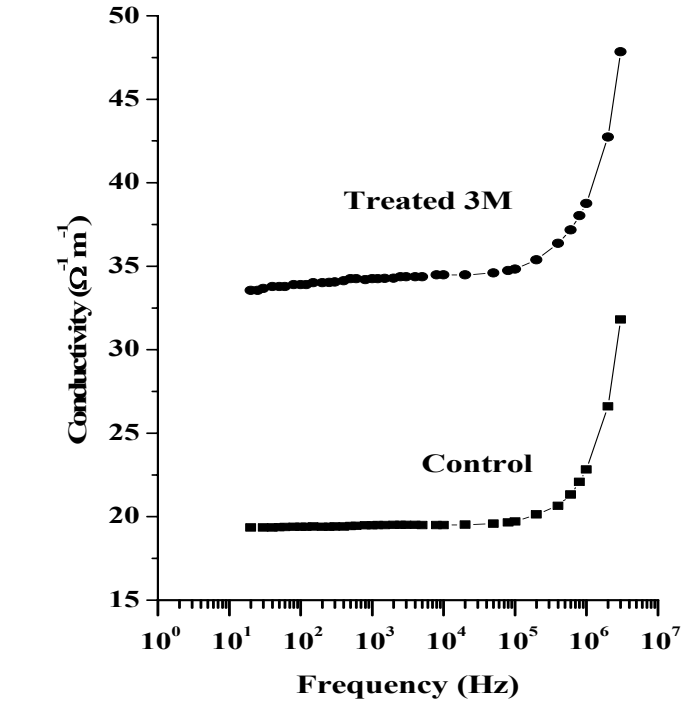
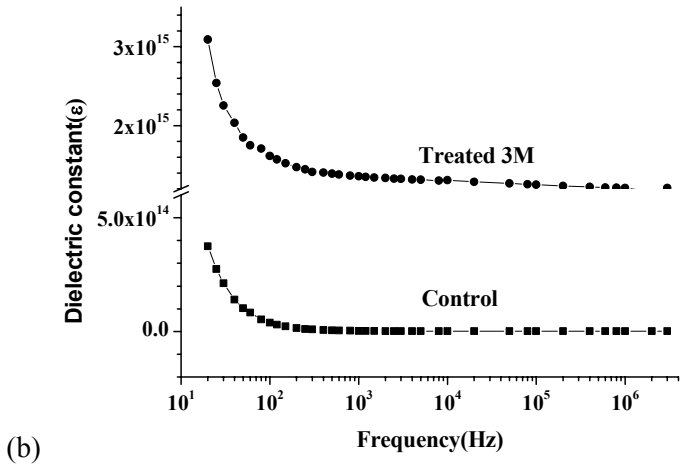


Fig. 1. Hemoglobin absorption spectra of rats exposed to lead for one, two, and three months treatment respectively as compared to control.



(a)



(b)

Fig. 2. Electrical conductivity σ (a) and dielectric constant ε (b) as a function of applied frequency in the range of 20 Hz to 3 MHz for hemoglobin solution (control ■) and after three months treated (•).

Table 2

Values of dielectric increment ($\Delta\varepsilon'$) and relaxation time (τ) for control and three months treated groups

Parameter / Sample	$\Delta\varepsilon'$	τ (ms)
Control	$(16\pm3.26)\times10^{14}$	2.0 ± 0.21
3 months	$(3.7 \pm 0.94)\times10^{14}$	3.5 ± 0.25

DISCUSSION

Lead has been recognized as a biological toxicant and different doses have been used to study lead-induced alterations. Absorbed lead following oral ingestion is carried via blood to soft tissues and 95 per cent of blood lead is transported on the erythrocytes as lead diphosphate [14]. This might be the reason of lead concentration increase in the blood following oral exposure to lead.

The results in Table 1 indicate that lead acetate ingestion induced a significant elevation of serum ALT, AST, and ALP levels at one, two, three months of lead treatment. Since aminotransferases (ALT and AST) are an important class of enzymes linking carbohydrate and amino acid metabolism, the relationship between the intermediates of the citric acid cycle is well established. These enzymes are regarded as markers of liver injury [43]. ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. It has been reported that [21] serum ALT was elevated significantly more than AST on lead exposure which indicates liver damage [38] and development of fibrosis [25]. Similarly [44] reported alteration in liver functions after chronic lead exposure. Lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes. It can also perturb protein synthesis in hepatocytes [15]. The observed decrease in protein content of plasma of rats treated with Pb may be due to decreased hepatic DNA and RNA [38]. It has reported [10, 21] a decrease in hepatic total protein content in response to lead intoxication. They attributed that to a decreased utilization of free amino acids for protein synthesis. It was noticed in the present investigation a significant increase in plasma cholesterol. It was found that administration of Pb to rats [2] elevates plasma LDL (low density lipoprotein) and reduces plasma HDL (High density lipoprotein). There is evidence that linking increased of serum cholesterol and LDL levels to a higher risk for developing coronary heart diseases [17].

A partial decrease in the intensity of the Hb absorption bands for lead treated animals was observed when compared to control. This indicates a partial loss of Hb molecule stability. Lead is known to interfere with heme and hemoglobin synthesis which affect erythrocyte morphology and survival [23].

Moreover, free radicals produced in the presence of heavy metals contribute to hemoglobin denaturing and precipitation, leading to anemia [39]. Interference in heme synthesis through inhibition of delta aminoleviolinic acid dehydratase resulting in increased delta aminoleviolinic acid which is one of the important biochemical effects of lead [28].

Dielectric measurements indicate that all studied samples have a dielectric dispersion in the frequency range of 20 Hz to 100 kHz which are called α-dispersion region. It is also noticed that the conductivity is frequency dependent and it is a mirror image to the change ε' with frequency.

The dielectric properties of Hb for treated rats showed that dielectric constant and conductivity have higher values than the control (untreated) which indicates a large increase of the surface charge density of the Hb molecules resulted from the formation of highly reactive molecular species. The generation of these highly reactive species, such as superoxide radicals ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and lipid peroxides (LPO) have been reported as lead induced effects.

Lead treated rats show a remarkable increase in the relaxation time of the Hb molecules. The results indicate a decrease of the free motion of the Hb molecules with increasing exposure time to lead. Moreover, since the dipole moment is directly proportional to the relaxation time, the increase in τ will cause an increase in the value of the dipole moment of the treated animals. Consequently, a higher electric conduction is expected as a direct response of the highly surface charge density.

Since the value of $\Delta\varepsilon'$ is a function of the perpendicular distance between the center and the axis of rotation of the Hb molecule, it is a function of the tilting angle of the Hb head group with the axis [13, 16, 37]. The increasing in the value of dielectric increment $\Delta\varepsilon'$ for 3 months treated rats indicates an increase in the perpendicular distance between the center and axis of the rotation of Hb molecules. The interpretation of this change suggests the change in the molecular shape and the structure of hemoglobin molecule.

CONCLUSION

The exposure to lead possess the potentials to induce hazardous biological effects in rats. The main damaging role of exposure to lead may be on the cellular membrane. The change in liver function and also the change in the shape and structure of hemoglobin molecule, which is the main component of RBCs, may damage organs such as liver and other critical organs.

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