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Lead Poisoning Chelation Therapy of *Angelica Archangelica*

[1]Shadia A. Fathey; [2]Abd El-Baset M.S.; [1]Fatma F. Abdel Hamid; [2,3]Bassem M.R. [4]Sabah M. Hassan and [2]Noha A. Abd EL-Latif

[1]Biochemistry Dept. Faculty of Science, Ain Shams University, Cairo, Egypt
[2]National research Center, Genetic Engineering and Biotechnology Division. 12622- Dokki, Giza, Egypt
[3]College of Applied Medical Sciences, Taif University, Taif, KSA
[4] Genetics Dep. Faculty of Agriculture, Ain Shams University, Cairo, Egypt

ABSTRACT

Lead can disturb cellular and molecular processes in the body and affect many organs and physiological functions. It can interfere with certain cellular signaling processes, the generation of action potentials in certain nerve cells and the function of a number of enzymes. Exposure to inorganic lead induces apoptosis. This study aimed to monitor the effect of elevated lead ions concentration on antioxidant enzymes and apoptotic related proteins. Animal study design was conducted to sixty male Sprague-Dawley rats divided into four groups, each contains 15 rats: G_I: Negative control group which represents rats did not receive lead through drinking water, G_{II}: Positive control group which represents rats received lead acetate (1000 ppm) through drinking water for 21 day. G_{III} : rats received lead acetate in drinking water for 21 days and then received *Angelica archangelica* roots extract for another 21 days with continuous exposure to lead acetate in water. G_{IV}: rats received lead acetate in drinking water for 21days and then received doses of dimercaptosuccinic acid (DMSA) for another twenty one days with continuous exposure to lead acetate in water.
Apoptotic related proteins P53 and Bcl2 were measured in liver tissues before and after application of chelators. Blood and liver lead concentration were measured to verify the chelation potency of chelators. Superoxide dismutase, glutathione peroxidase and catalase activities were determined for all groups to detect the effect of lead ions on the antioxidant enzymes activity. Thiol groups, malondialdehyde concentrations and auto-oxidation rate of hemoglobin were detected to show the effect of lead poisoning on animals.
Results showed a reduction in both P53 and Bcl2 in animals received chelation therapy as compared with lead intoxication group (positive control) with decrease in the activity of antioxidant enzymes in animals received chelation therapy as compared with lead intoxication group. We conclude that both chelators (DMSA and *A. archangelica*) have a role in reducing the hazards effects of lead poisoning, which results in oxidative stress and free radicals formation, decreasing the activity of antioxidant enzymes (SOD, GPX and catalase), the concentration of lipid peroxidation products (malondialdehyde) and the concentration of apoptotic related proteins (P53 and Bcl2) and normal auto-oxidation rate for hemoglobin with increasing in thiol group concentration.
KEYWORDS: apoptosis – P53 – Bcl2 – lead – oxidative stress.

INTRODUCTION

Lead (Pb) is one of the most toxic heavy metals may causing health problems such as behavioral anomaly learning disabilities and seizures **(Bulut and Baysal, 2006)**. It is classified as a Group B2 carcinogen (possible human carcinogen) by the International Agency for Research on Cancer **(Apostoli and Boffetta, 2000)**. Lead is not number one metal of the periodic table but its usage has made it number one. Lead became popular because of its dense, ductile, malleable and corrosion resistant properties **(Florea and Busselberg, 2006**). These properties have made lead useful in building materials, pigments to glaze ceramics, water pipes and glass, paints and protective coatings, acid storage batteries and gasoline additives. Due to its wide applications and usage, exposure of humans to lead and its derivatives in day-to-day life is unavoidable. Lead poisoning is one of the oldest and the most widely studied occupational and environmental hazards **(Flora *et al*., 2006).**

Lead binds to sulfhydryl and amide group components of enzymes, altering their configuration and diminishing their activities. It may also compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium **(Flora *et al*., 2007).** Lead produces a range of effects, primarily on the haematopoietic system, nervous system and kidneys **(Jaffe, 1995).**

Chelating agents are organic or inorganic compounds capable of binding metal ions to form complex ring-like structure called ‘chelates’. Chelating agents possess “ligand” binding atoms that form either two covalent linkages or one covalent and one co-ordinate or two co-ordinate linkages in the case of bidentate chelates. Bidentate or multidentate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal **(Andersen, 1999).**

***Corresponding Author:** Bassem M. Raafat Assistant Professor of Biophysics Genetic Engineering and Biotechnology Division, National Research Center, Cairo, EGYPT ,bassemraafat@hotmail.com

Chelation therapy is the administration of chelating agents to remove heavy metals from the body. For the most common forms of heavy metal intoxication those involving lead, arsenic or mercury. The chelating agent may be administered intravenously, intramuscularly, or orally, depending on the agent and the type of poisoning Some common chelating agents are ethylenediaminetetraacetic acid (EDTA), 2,3-dimercaptopropanesulfonic acid (DMPS), thiamine tetrahydrofurfuryl disulfide(TTFD) and 2,3-dimercaptosuccinic acid (DMSA). Calcium-disodium EDTA and DMSA are only approved for the removal of lead by the Food and Drug Administration (FDA). These drugs bind to heavy metals in the body and prevent them from binding to other agents. They are then excreted from the body **(Bridges, 2006).** A major advantage of DMSA is that lead is not redistributed to the brain and other vital organs after its therapy in rats intoxicated with lead **(Cory-Slechta, 1988; Flora *et al*., 1995).** Animal studies suggest that DMSA is an effective chelator of lead concentrated in soft tissue but it is unable to chelate lead from bones **(Gurer *et al*., 1998). Ercal *et al*., (1996)** indicated that lead induced oxidative stress responded moderately to the treatment with DMSA accompanied by reduction in lead concentration from blood and soft tissue. DMSA for being an antioxidant and a strong lead chelator has been shown to deplete significantly lead from hippocampus leading to recovery in the oxidative stress and apoptosis induced by lead **(Zhang *et al*., 2004).**

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms **(Green, 2011).** Characteristic cell changes (morphology) may occur include blebbing, loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation **(Alberts *et al*., 2008).** The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions. It should be stressed that apoptosis is a well-defined and possibly the most frequent form of programmed cell death, but that other, non-apoptotic types of cell death also might be of biological significance **(Leist, 2001).**

MATERIALS AND MAETHODS

The experiments were performed on sixty male Sprague-Dawley rats weighing 200 g (± 20 g). 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 National Research Center (NRC).

Animals were subdivided into four groups each contains 15 rats: G_I: Negative control group which represents rats did not receive lead acetate through drinking water, G_{II}: Positive control group which represents rats received lead acetate (1000 ppm) through drinking water for 21 day. $Group_{III}$: rats received lead acetate (1000 ppm) in drinking water for 21 days and then received *A. archangelica* roots extract for another 21 days with continuous exposure to lead acetate in water. G_{IV}: rats received lead acetate (1000 ppm) in drinking water for 21days and then receive doses of dimercaptosuccinic acid (DMSA) for another twenty one days with continuous exposure to lead acetate in water.

Drinking water was prepared as 1000 ppm lead acetate solution. Acidification of water is essential for dissolving lead acetate in water so 1 ml of conc. hydrochloric acid was added per liter of deionized water. 1000 ppm lead acetate dose is chosen to give a desired level of toxicity in blood and liver.

A. archangelica roots were crushed. Boiled distilled water was added to 15gm of this powder. After cooling, juice was filtered three times using Wattman filter no. 4. The obtained solution was used in a base of body weight as 5 ml for each kg body weighs **(El-Gohary *et al*., 2009)**. 50 mg/kg body weight of DMSA was orally administered to rats **(Lee *et al*., 1995).**

Blood lead level was determined according to Salvin, (1968) using a Pye-unicum SP 90 series atomic absorption spectrophotometer. Lead concentration in rats liver was determined using dry ashing technique (**Yeager *et al*., 1971).** Determination of apoptosis-related proteins, p53 and bcl2, was performed on liver tissues using ELISA technique **(Tijssen, 1985).** P53 protein was determined by TiterZyme® EIA p53 Enzyme Immunometric Assay Kit, cat.No.900-117117. Bcl-2 was determined by Total Bcl-2 Enzyme Immunometric Assay Kit Assay designs kit package cat. No.900-133. Blood super oxide dismutase (SOD) and glutathione peroxidase (**GPX**) activities were carried out by a RANDOX kit package according to **McCord and Fridovich (1969) and Wendel (1981)** respectively**.** Plasma of catalase activity was performed according to Aebi (1984) while the concentration of malondialdehyde was carried out by a coloremetric method using Biodiagnostic kit package **(Satoh, 1987).** Estimation of total and protein- nonbound sulfhydryl groups in tissue was carried out by Ellman's reagent procedure **(Sedlak and Lindsay, 1967).** Autooxidation rate measurements in hemoglobin solution was performed according to **William *et al* (1982).**

Samples were collected for the biochemical and molecular investigations by a suitable syringe for this purpose and stored in tubes containing heparin at- 70°C until the time of analysis. During sample preparation, care was paid to avoid contamination, therefore, every item from the moment of sampling until analysis was regarded as potential source of contamination and was checked not to contain or leach detectable amount of any

contaminant. Sample collection was performed after finishing the 21 days of lead exposure with or without chelation therapy application.

Observed data were statistically analyzed by using of SPSS program. All tests were two-tailed

RESULTS

After administration of lead acetate only through drinking water (G_{II}), lead acetate in drinking water concomitant with *A. archangelica* root water extract (G_{III}) and lead acetate in drinking water concomitant with DMSA (G_{IV}) for twenty one day compared to control, the concentration of lead in blood and liver was determined.

Table (1): Lead concentration in blood and liver of rats (Mean ± S.D.)

Groups	Blood Lead Conc. (µg/dl)	Liver Lead Conc. (µg/100 g wet wt)
G_I (n = 15)	2.14 ± 0.14	5.25 ± 0.19
G_{II} (n = 15)	66.12 ± 4.89 [a]	125.14 ± 9.45[a]
G_{III} (n = 15)	12.12 ± 1.47[a,b]	23.47 ± 1.98 [a,b]
G_{IV} (n = 15)	24.57 ± 4.14 [a,b]	64.14 ± 5.14 [a,b]

a significant difference (P< 0.001) compared to G_I
b significant difference (P< 0.001) compared to G_{II}

Table 1 shows that administration of lead acetate for 21 days increased both liver and blood lead concentration compared to negative control group (G_I)(p<0.001). Dramatic significant reduction (p<0.001) was recorded in rats received chelator but *A. archangelica* extraction showed higher chelation potency regarding lead concentration in both blood and liver (12.12 ± 1.47 and 23.47 ± 1.98 µg/dl, respectively). Attention should be drawn to blood and liver lead concentration in rats treated with DMSA; its concentration reduction value was lower than that recorded in rats treated with herbal chelation (64.14 ± 5.14 *vs* 23.47 ± 1.98).

Table (2): Apoptotic related proteins (P53 and Bcl2) in rats liver of all groups (Mean ± S.D.)

Groups	P53 (pg/ml)	Bcl2 (pg/ml)
G_I (n = 15)	4.52 ± 0.35	4.38 ± 0.35
G_{II} (n = 15)	6.88 ± 0.52[a]	5.85 ± 0.30[a]
G_{III} (n = 15)	5.32 ± 0.48[a,b]	4.82 ± 0.35[a,b]
G_{IV} (n = 15)	5.64 ± 0.48[a,b]	4.99 ± 0.41[a,b]

a significant difference (P< 0.001) compared to G_I
b significant difference (P< 0.001) compared to G_{II}

Table 2 shows a significant decrease in both p53 (P< 0.001) and bcl2 (P< 0.001) after application of *A. archangelica* and DMSA.

Table (3): Blood SOD, GPX and plasma catalase activities in all groups (Mean ± S.D).

Groups	SOD (U/ml)	GPX (U/ml)	Catalase (IU/L)
G_I (n = 15)	55.14 ± 4.58	4521.08 ± 384.05	415.87 ± 45.62
G_{II} (n = 15)	186.14 ± 11.25[a]	9562.52 ± 625.14[a]	528.89 ± 47.07[a]
G_{III} (n = 15)	68.47 ± 5.47[a,b]	5423.52 ± 525.47[a,b]	433.48 ± 44.13[b]
G_{IV} (n = 15)	89.47 ± 6.41[a,b]	6325.85 ± 541.25[a,b]	442.06 ± 31.09[b]

a significant difference (P< 0.001) compared to G_I
b significant difference (P< 0.001) compared to G_{II}

In intoxicated animals (G_{II}), antioxidants enzyme activites significantly increase (P< 0.001) compared to the control group (G_I). antioxidant enzymes activities were decreased after administration of chelators showing the lowest decrease in case of *A. archangelica* (P< 0.001) compared to G_{II} (Table 3).

Table (4): MDA, total and protein- non bound sulfhydryl groups concentrations in liver tissues of all rats groups (Mean ± S.D.).

Groups	MDA (nmol/g tissue)	Total SH (µmol/g tissue)	protein- non bound SH (µmol/g tissue)
G_I (n = 15)	10.88 ± 1.03	283.90 ± 23.55	0.69 ± 0.05
G_{II} (n = 15)	14.66 ± 1.10[a]	215.13 ± 19.71[a]	0.53 ± 0.04[a]
G_{III} (n = 15)	10.66 ± 0.84[b]	285.96 ± 22.28[b]	0.72 ± 0.06[b]
G_{IV} (n = 15)	11.96 ± 0.61[a,b]	263.80 ± 23.95[b]	0.66 ± 0.06[b]

a Significant difference (P< 0.001) compared to G_I
b Significant difference (P< 0.001) compared to G_{II}

In the group of animals treated with lead only, the concentrations of MDA increased (14.66 ± 1.10 nmol/g tissue), Significant decrease ($p< 0.001$) in MDA was detected after application of chelators (G_{III} and G_{IV}). On the other hands, total sulfhydryl groups and protein- non bound sulfhydryl groups were decreased in the group of animals treated with lead (215.13 ± 19.71 and 0.53 ± 0.04 respectivelly) then increased after chelators treatment specially the *A. archangelica* chelator (Table 4).

Figure 1 shows the auto-oxidation rate as a plot between the met-hemoglobin formation rate against time. The lower the slope is the lower rate of oxidized form of hemoglobin produced. Animals received lead acetate in drinking water with no chelatoin therapy showed the highest rate of auto-oxidation. It is obviously shown that animals treated with *A. archangelica* extraction revealed lower rate of auto-oxidation that reflected the rate by which hemoglobin is converted to non-functional hemoglobin. This rate was even lower than that of animals hemoglobin treated with DMSA

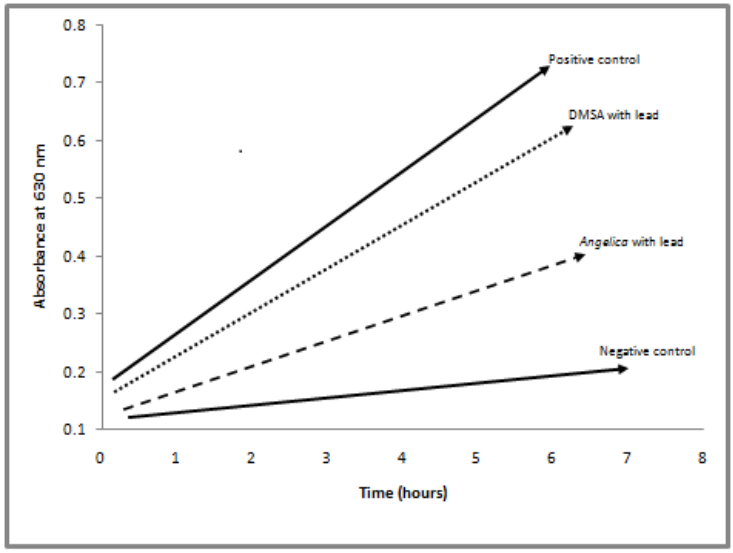

Figure (1): Hemoglobin auto-oxidation of rats groups

DISCUSSION

Lead can disturb cellular and molecular processes in the body and affect many organs and physiological functions. It can interfere with certain cellular signaling processes, the generation of action potentials in certain nerve cells and the function of a number of enzymes **(Rana, 2008)**. It is well established that the main toxic mechanism of lead is through the elevated rate of reactive oxygen species (ROS) formation **(Yedjou *et al*., 2006 & Yedjou *et al*., 2010).** Yiin and Lin **(1995)** demonstrated marked enhancement in malondialdehyde (MDA) as a result of incubation of linoic, linolenic, and arachidonic acid with lead. This finding was proven by many other studies, which have pointed to either elevated lipid peroxidation or decreased intrinsic antioxidant defense in various tissues of lead-exposed animals **(Shafiq-ur-Rehman et al., 1995; Sandhir and Gill, 1995)**

The clinical importance of the herbal drugs has 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. *A. archangelica* is one of the famous and oldest plants that have been used therapeutically for centuries. It is a natural antioxidant. *A. archangelica (*European *A. archangelica*) is a member of a widely cultivated *A. archangelica* herb. It has been used in traditional medicine as a remedy for various disorders such as: headaches, backaches, asthma, skin disorders and digestive disorders **(El-Gohary, 2009).**

In this study there is a significant ($p<0.001$) elevation of superoxide dismutase, glutathione peroxidase and catalase activity as well as elevation in the concentration of malondialdehyde in animals received lead acetate in the drinking water for twenty one days which is an evidence of highly formation of reactive oxygen species. This production rate was significantly ($p<0.001$) reduced after application of both chelators.

Lead binds with sulfydryl groups and therefore may change the structure and function of certain proteins and enzymes **(Rana, 2008)**. An additional evidence of highly ROS formation rate is the significant decrease ($p<0.001$) in the concentration of total and non bound protein sulfhydryl (thiol) groups in animals received lead acetate in the drinking water for twenty one days compared to control group. Thiol group's concentration was significantly increased ($p< 0.001$) after application of both chelators as compared to positive control group. *A. archangelica* water extraction revealed the best results and this is may be due to the double impact role of the extraction as a chelator **(El-Gohary *et al*., 2009)** and antioxidant **(Yeh *et al*., 2003).**

There are several studies indicated that apoptosis might be associated with lead-induced oxidative stress and DNA damage **(Yedjou *et al*., 2006 & Yedjou *et al*., 2010).** Exposure to inorganic lead induces apoptosis in rod photoreceptor cells **(Fox *et al*., 1998)**, neuronal cells **(Shabani and Rabbani, 2000)**, hepatocytes **(Columbano *et al*., 1985)** and macrophages **(Cheng *et al*., 2002)** but not in human mononuclear cells **(De la Fuente *et al*., 2002)**. In lead-induced apoptosis, the mitochondria play a crucial role. Since lead mimics calcium, calcium overload may trigger apoptosis. Calcium and lead both depolarize rod cell mitochondria due to the opening of the permeability transition pore (PTP), resulting in the cytochrome C release, caspase activation and apoptosis. (PTP) does not open due to oxidative stress **(He *et al*., 2000).**

The rate of apoptosis is controlled by the balance between proteins that activate processes resulting in such death and other proteins that act to inhibit these processes. Thus, for example, the *Bcl-2* proto-oncogene was originally identified on the basis of its activation by chromosomal translocation in non-Hodgkin B cell lymphomas **(Tsujimoto *et al*., 1985)** and was subsequently shown to protect a wide variety of different cell types from programmed cell death or apoptosis. Conversely, the p53 anti-oncogene protein, as well as inhibiting cellular proliferation, can also stimulate programmed cell death. It is clear therefore that Bcl-2 and p53 represent proteins with opposite effects on the rate of apoptosis (**Agarwal *et al*., 1998).** Moreover, it appears that Bcl-2 can specifically inhibit p53-dependent apoptosis. Thus, although over expression of p53 can induce apoptosis in different cell types, this is prevented by over expression of Bcl-2 **(Chiou *et al*., 1994; Marcellus *et al*., 1996).** In this regard, it is of interest that high levels of p53 are associated with low levels of Bcl-2 and *vice versa*, both during normal rat development **(Ibrahim *et al*., 1997)** and in different types of tumors **(Harn *et al*., 1996; Lee *et al*., 1996).**

In this study, results showed highly significant increase ($p< 0.001$) in concentrations of apoptotic related proteins, p53 and bcl2, in groups of animals received lead acetate in the drinking water for twenty one days compared to control group. The concentration of these proteins was significantly decreased ($p< 0.001$) after application of both chelators as compared to positive control group It is obviously noticed that *A. archangelica* water extract revealed the best chelation potency as compared to DMSA groups.

Lead interferes with heme biosynthesis **(Rana, 2008)**. A normal physiological function of hemoglobin is the reversible binding of oxygen which can occur with the heme iron in the reduced (ferrous) state. In normal case, hemoglobin undergoes auto-oxidation to met-hemoglobin (oxidized form) at a rate of about 3 % per day. Normally the methemoglobin can be restored to a functional form through reductive processes that take place in the red cell to maintain a maximum level of met-hemoglobin about 1 % of the total hemoglobin. Met-hemoglobin reductase is the enzyme involved in this process **(Jaff and Neumann, 1964).**

The rate of normal dissociation of oxyhemoglobin to met-hemoglobin is highly dependent on the tertiary and quaternary structure of hemoglobin molecule. The quaternary structure, which is necessary for cooperative functional aspects of oxygen binding of the hemoglobin molecule, is also related to its molecular stability **(Rachmilewitz *et al*., 1974).**

The higher rates of hemoglobin auto-oxidation in animal received lead for a long time may be due to the metal-globin complex formations which play an important role in changing the stoichiochemical structure. Role of the higher concentration of free radicals formed in the elevated blood lead levels has not to be away from the change of normal oxy-hemoglobin to a non-functional hemoglobin derivative (met-hemoglobin) **(William *et al*., 1982).**

In this work, auto-oxidation rate plotting (Fig.1) recorded the highest rate in group II which receive lead in drinking water followed by DMSA administration with continuous lead poisoning when compared to the auto-oxidation rate that plotted to groups treated with the water extraction of *A. archangelica* roots. This result is in agreement with the correlation found in this group between blood lead level and the activity of antioxidant enzyme (SOD, GPX and catalase). Both results are due to the formation of larger amounts of free radicals because the elevated lead concentration.

In conclusion, both chelators (DMSA and *A. archangelica)* have a role in reducing the hazards effects of lead poisoning, which results in oxidative stress and free radicals formation, by decreasing the activity of antioxidant enzymes (SOD, GPX and catalase) as well as lipid peroxidation and reducing the concentration of apoptotic related proteins (p53 and Bcl2) with normal autooxidation rate for hemoglobin. Thiol group concentration were increased after application of chelators

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