Review Article

Effects of Vitamin C on Lead Induced Developing Thymus in Mice: A review

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Abstract:

The present review deals with effects of vitamin C on lead intoxicated developing thymus in mice .In the world of environmental health and medicine, lead exposure remains one of the most important problems in terms of prevalence of exposure and public health impact. Previous research has shown that every level of lead exposure is dangerous to animals and humans. Thus lead toxicity continues to be a leading environmental issue. Little is known about the persistent Immunotoxic effect of Pb when administered during specific stages of embryonic development. A variety of toxic effects caused by lead exposure during gestation and lactation have been reported in human and animal studies. Our immune system is our most powerful weapon against disease. Fetal and early postnatal life represents critical periods in vertebrate immune system development. Disruption of such development by Immunotoxic chemical exposure has been widely described in experimental animal model. The available animal data suggest the potential immune function in humans exposed immunotoxicants during fetal and early postnatal life. Administration of vitamin C results in improvement in several components of human immune response. Vitamin C regulates the immune system because of its antioxidant properties and its role in collagen synthesis required for stabilization of epithelial barriers. It plays a role in phagocytic function and has an immunostimulatory effect on lymphocyte cells. Limited information is available regarding the possibility of inhibited postnatal immune capacity in humans as a result of pre and postnatal immunotoxicant exposure. To fulfill this lacuna an attempt has been taken to gather the significant information on deformities in immune system due to immunotoxicant exposure during pre and postnatal development.

Keywords: Lead, Thymus, Postnatal, Immunotoxicity, Vitamin C

1.0 Introduction:

The natural environment is necessary for the existence of present and future generation (Bogner, *et al.,* 2008; Andrew, *et al.,* 2007) and has provided all the basic needs of human societies thought history (Smith and Curnus, 2008). Lead remains a significant occupational and public health problem. Despite decades of research, lead toxicity also remains one of the most studied subjects of all with in the fields of environmental health and medicine. The majority of the industries associated with high lead exposure have made advances in their control of occupational exposure. However, cases of unacceptably high exposure and even of frank lead poisoning are still seen, predominantly in the demolition and tank cleaning industries. Modern research findings indicate that lead may be toxic at levels previously thought to have no harmful effects. In most industries blood lead levels have come down below levels at which signs or symptoms are seen and the current focus of attention is on the sub-clinical effects of exposure. Inevitably there is pressure to reduce lead exposure in the general population and at work places, but any legislation must be based on a genuine scientific evaluation of the available evidence (Gidlow, 2004).

Lead is the metal, which has been associated with human activities from the past. Levels of lead content in various media have been coupled with data for lead intake and absorption in the human body, for both children and adults are affected not only morphologically but also anatomically and histologically. Various organs also show critical damage (Pizzol, *et al.,* 2010). The general population could be significantly exposed owing to poorly glazed ceramic ware, food canning, battery cosmetic and mining industry, contaminated water by lead and motor vehicle deposition. It was an important cause of morbidity and mortality during the Industrial Revolution and effective formal control of lead workers did not occur until the pioneering occupational health work of Lane (1949). In view of the long history of lead's toxicity and the extensive publications one would think that lead exposure is controlled up to a major extent and lead poisoning was merely a historical entity. Unfortunately, this is not the case; there are still industries in this country where clinical lead poisoning occasionally still occurs (Levin, *et al.,* 1997).

2.0 Effect of Lead on the Body:

In spite of continuously exposure to lead and absorption through various routes it has no known biological function in the body, and is highly toxic and accumulates in various tissues of human body such as bone, blood, kidney, liver and thymus (Baldwin and Marshall, 1999). Peoples have known about the poisonous effects of lead from 200BC. The lead has been used comprehensively for both industrial and domestic applications for hundreds of years. As exposure to lead in work places and in the environment is profoundly regulated now, there are few cases of acute lead poisoning. The first deliberation of toxicity is exposure of an organism to a toxic substance. The major routes of accidental or intentional exposure to toxicants by humans and other animals are the skin, the lungs (inhalation, respiration, pulmonary route), and the mouth (oral route). The way by that a toxic substance is introduced into a complex system of an organism is strongly dependent upon the physical and chemical properties of the substance. Lead have both physical and chemical properties to enter and affect the human body A man exposed to lead not only at work places, but also from lead based paint in older housing and from soil, that is still persists in the community. From the perspective of human reproduction, lead is known to cause a number of adverse consequences in both men and women. Effects in women include infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery (Winder, 1993).

Lead in the blood has a half-life of around 25 days and in tissue its half-life is about 40 days (Hu, 1998; Williams, *et al.,* 1999). Due to this, blood lead levels are not very useful as an indicator of how-much lead exposure an individual has undergone, as they only show recent exposure (Timbrell, 1995; Williams, *et al.,* 1999). However, in bone lead has a half-life of 25 years or more, and it is possible to estimate past exposure to lead by X-ray (Agency for toxic Substances and Diseases Registry, 1997). Lead is excreted from the body mainly in the urine, but also in the feces, and small amounts also appear in hair, nails, sweat, and saliva and breast milk. By these exposure media lead enters in the body and causes hematological, gastrointestinal, and neurological dysfunction in adults and children. Young children are particularly affected by lead poisoning as they absorb greater amounts from the gastrointestinal tract. Severe or prolonged exposure may also cause chronic nephropathy, hypertension, and reproductive impairment. Lead inhibits enzyme, alters cellular calcium metabolism, and stimulates synthesis of binding proteins in kidney, brain and bone, and slowdown nerve conduction. Less severe exposure to lead, designated by blood lead levels, has been implicated in poor pregnancy outcome, impaired neurobehavioral development, reduced stature in young children, and higher blood pressure in adults. Acute high lead exposure can cause serious physiological effects. Effects of lead exposure vary according to exposure, timing and levels, and other factors, and some effects may be latent. The pathological effects of lead on the renal, nervous, reproductive, endocrine, and immune systems have been reviewed. Emphasis is placed on reported subclinical effects due to chronic, low-level lead exposure. The crucial issue of whether subtle behavioral, intellectuals, and developmental impairment occurs in young children, as a result of lead-induced CNS damage is discussed in detail. This issue remains unresolved. Further studies are needed in order to determine the long-term health effects of continuous, low-level lead exposure (Damstra, 1977).

Little is known about the effects of lead on reproductive performance and postnatal development following chronic, low-level exposure. Kimmel, *et al.,* (1976) exposed female rats chronically to lead acetate via the drinking water (0.5, 5, 50, 250 ppm) from weaning through mating, to lactation. Vaginal opening was delayed 1-2 weeks but estrous cycle and pregnancy rates were normal in those females that are exposed to 50 and 250 ppm lead. No teratogenic effects and no significant increase in fetal resorption were observed, although exposure to 250 ppm lead acetate caused a slight, but no significant increase in fetal resorptions. The lead-treated animals produced litters of normal numbers, but the offspring from the 50 and 250 ppm groups weighed less at weaning and showed delays in physical development. Reiter, *et al.,* (1975) also observed developmental delays in rat offspring exposed to 50 ppm lead from gestation to lactation. Whether these delays in development were the result of a direct effect of lead on the nervous system of the pups or reflect secondary changes (malnutrition, hormonal imbalance, etc.) is not clear. Whatever the mechanisms involved, these studies do suggest that low-level chronic exposure to lead might induce postnatal developmental delays.

Several studies in animal models have suggested that lead may interfere with various aspects of the immune response. Lead has been reported to

result in an increased susceptibility to infection in mice and rats. Various factors may be involved in producing this enhanced susceptibility to infection. Smith *et al.,* (2008) results show that dietary lead concentration did not affects body weight gain and food consumption but there are significant increase in fecal lead concentration and total fecal lead output. There work also shows dose depend increase in tissue lead concentration. Jiao *et al.,* (2010) also reported that there were no significant differences in body weight of control and lead treated animals but continuous exposure of lead increase blood lead levels in all treated animals. A significant increase in the serum alkaline phosphate activity and increase in the level of serum bilirubin content indicate serious hepatic damage if lead is exposed for 7 days. Hemoglobin also decreased due to above treatment because of breakdown of haemoglobin in its downstream products or because the process of hemoglobin biosynthesis may have been affected (Kilikdar *et al*., 2011). Sharma *et al.,* (2011) also demonstrated that lead exposed swiss albino mice show the loss of body weight and significant decrease in RBC count, WBC count, Hb level and serum total protein count. Animal also show the increase in serum alanine transaminase, aspartate aminotransferase, and creatinine and cholesterol level. Lead, a common environmental contaminant that has no known beneficial or desirable nutritional effect, has been found to produce a wide range of adverse effects that involve several organic systems and biochemical activities (Patrick, 2006).

3.0 Thymus Development:

Most embryological evidence at present favours the view that the thymic epithelium is derived from both the ectoderm and the endoderm of the third and the fourth branchial grooves and pharyngeal pouches. The rodent thymus develops from the endoderm of the 3rd and 4th pharyngeal pouches and surrounding mesenchyme (Dijkstra and Sminia, 1990). As development progresses, the thymus along with the thyroid and parathyroid, sharing the same pharyngeal pouch origin, migrate caudally. They separate around day 15 when the thymus migrates into the thorax. Embryonic thymic remnants can give rise to ectopic thymic tissue in the neck, thyroid and parathyroid glands (Suster and Rosai, 1992). The thymus is the first of the lymphoid organs to be formed and grows immediately after birth in response to postnatal antigen stimulation and the demand for large numbers of mature T cells. Genetic factors also influence the age of onset,

rate and magnitude of thymus dependant immunological function.

3.1 Normal Anatomy:

Located in the upper part of the thorax, the thymus is quite large in the young animals, but as the animal grows, the gland atrophies. The mammalian thymus is located in the mediastinum, anterior to the major vessels of the heart, and ventral to the base of the heart and aortic arch, with variable extension of one or both lobes into the cervical region in the rat (Haley, 2003). In mice thymus is situated anterior to the pericardial membrane. The thymus consists of two distinct lobes. A connective tissue named isthmus, connect the both lobe of the thymus. Each lobe is covered by a thin connective tissue capsule and in most species lobes are divided into several lobules of different shape, size and orientation. There is no sublobulation in the mouse. Evidence for a functional cervical thymus in mice has been reported (Terszowski, *et al.,* 2006).

3.2 Histology:

Thymus is partly an endocrine gland and partly a lymphoid structure. According to various lymphoid organs the thymus is histologically most consistent in different animal species. It is an epithelial organ. It's each lobule divided into two regions outer one is cortex and inner one is medulla. Cortical region is dense to epithelial cell in comparison to medullary region. Both regions are separated by a vascular region named corticomedullary zone. The epithelial cells form an open framework containing predominantly T lymphocytes, smaller populations of B lymphocytes and plasma cells and scattered populations of other cells such as endocrine cells. It is divided into a morphologically distinct cortex and medulla separated by a vascular Corticomedullary zone. The medulla also contains reticular cells and the unique *"Hassall's Corpuscle"* which are spherical structures composed of concentric layers of spindle shaped cells whose function is unknown.

3.3 Epithelial Stroma:

The bulk of the supporting framework in the thymus is composed of the network of epithelial-reticular cells (Banks, 1993). Epithelial cells in the subcapsular region of the thymus form one or two cellular layers. In the outer cortex and ensheathing blood vessels, epithelial cells are thin and sheet like, but elsewhere they assume a stellated appearance. Epithelial cell populations differ in structural characteristics, antigen expression and the hormone synthesis capacity. These cells are

divided into four subcategories, sub capsular cortical cells, inner cortical cell, medullary cells and Hassalls corpuscles on the basis of immunohistochemical techniques (DeWaal, *et al.,* 1997). Number of antigenic epithelial population is decrease with the age of mice. More immunohistochemical studies are provided by Greaves (2000); Kuper, *et al.* (1995); and Suster and Rosai, 1992).

3.4 Capsule:

Each lobe of thymus is covered by a thin capsule which divides the lobe of thymus in several lobules of different size and orientations by septae. Capsule is made up by outer collagen fiber layer and inner reticular fiber layer and occasionally in between, clusters of lymphocytes are found. Supporting framework of thymus is made up by epithelial reticular cells network (Bruijntjes, *et al.,* 1993; Elmore, 2006).

3.5 Cortex:

In the cortex, massive numbers of densely packed small lymphocytes predominate, occupying the interstices of the epithelial reticulum, which in histological sections largely obscure and forming about 90% of the total weight of thymus. Thymocytes undergo mitosis in all cortical zones as the clones of differentiating T cells mature, gradually moving deeper in the cortex. Histologically, the darkly staining cortex contains densely packed, small, immature lymphocytes; in the sub capsular cortex region there are some active lymphocytes found which have a round to oval nucleus with one or two well known nuclei and basic cytoplasm. A slope of small but active cell comes from the outer cortex to the corticomedullary junction.

3.6 Corticomedullary Junction:

Corticomedullary junction is composed by overflowing blood vessels, slight perivascular connective tissue and mature and immature T-lymphocytes. Arterioles of overflowing blood vessels are divided into capillaries and extend into cortical and medullary region. In this region B-lymphocyte and plasma cells are also found which increase with the age of animal.

3.7 Medulla:

The central part of the lobule which has less epithelial cells density in comparison to cortex is known as medulla. In this region we found the more mature T-lymphocyte, well known epithelial cell, Hassalls corpuscles, mixed macrophages, immature B-lymphocytes and few myoid cells. T-lymphocyte are larger, mature, well stained and have more cytoplasm in this region comparison to cortical region T-lymphocyte.

3.8 The Corticomedullary Ratio:

The area of cortex and medulla is known as corticomedullary ratio. Early in life cortex is large in comparison to medulla it means ratio is more than one but later in life (after involution) cortex decrease and medulla increase than corticomedullary ratio decrease (less than one) (Elmore, 2006). Therefore we can say that corticomedullary ratio depended upon the age of animal. Tryphonas, *et al.,* (2004) provided morphometric measurements of histological sections of the thymus in control Sprague–Dawley rats. By their method, the average cortico-medullary ratio was determined to be 4.4 to 4.7 (30 and 90 day-old males) and 3.9 to 6.3 (30- and 60 day-old females).
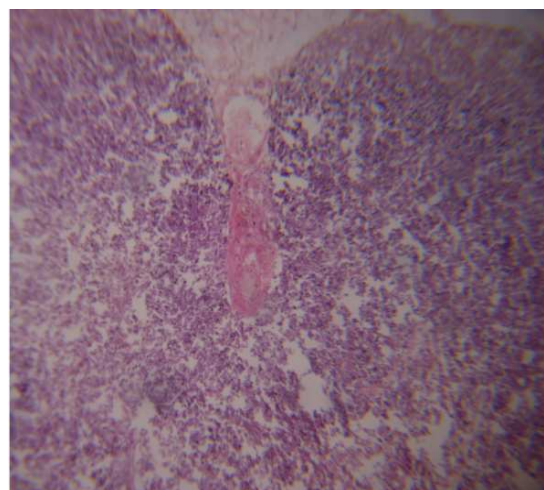

Fig. A: Photomicrograph of thymus shows cortex and medulla composed of epithelial cells. At this stage more epithelial cells are present in cortical region in comparison to medullary region. (Original magnification, x 10; hematoxylin and eosin stain).

4.0 Thymic involution:

As we know, the thymus is a lymphoid organ; it develops gradually parallel to other organs till puberty. After puberty several changes occur in its morphology and cellularity. This phenomenon is known as thymic involution. During thymic involution cortical and medullary region reduce; there is also a decrease in number of cortical lymphocytes and increase in tangible body macrophages. In histological view involution is reduction in the size, decrease cortical lymphocyte, irregular cortex and demarcation of corticomedullary zone, increase the perivescular space, perivascular B lymphocyte and plasma cells.

The thymus body ratio is greatest perinatally but the organ continues to increase in absolute size until about puberty, after which it tends to gradually decline. According to Kuper, *et al.,* (2002) when there is a decrease in thymic size and cellularity one should use the term "reduce number of cortical lymphocyte and increase number of macrophages". These changes may be identified as "atrophy or involution". Normal development, histology, and function of the thymus have been reported previously (Kuper, *et al.,* 1992; Pearse, 2006). The use of standardized descriptive nomenclature with respect to thymic pathology is addressed (Haley, *et al.,* 2005; Elmore, 2006).

4.1 Nonproliferative Morphological Changes in the Thymus Factors Effecting Thymic Cellularity:

A direct correlation exists between thymic microenvironment and developing T cells. Multiple factors and conditions result in an alteration in the cellular density and cellular composition of the thymus. Most commonly recognized is a decrease in lymphocytes resulting from a range of background physiological influences and the immunosuppressive effect of xenobiotics. Inadequate nutrition, social stress also affects thymic involution. The histological appearance of the thymus under these varied conditions is similar, since the end point is the reduction in cortical lymphocytes, and shrinkage of the thymic lobules (Schuurman, *et al.,* 1994). During involution there is an increase the number of apoptotic bodies, tangible body macrophage and lymphocyte depletion. After involution medulla has a higher cellular density than the cortex. Factors such as stress and toxicity can simultaneously be superimposed on the normal ageing process of lymphocyte reduction. Consequently, the distinction between atrophy and involution in older animals can be problematic.

4.2 Age Associated Effects/Involution:

Animals age play an important role in the level of cellularity of the thymus and its overall histological changes. Physiological involution reflects the change in function of the thymus from lymphocyte production to recirculation. In rodent thymus develops gradually parallel with gonads but after puberty or gonadal development it decreases in size. It may be due to increased circulating levels of sex steroid hormones. Gonads play a role in thymic involution in both sexes (Grossman, 1985).

There are multiple pathways involved in lead effects on male reproductive system. It is unclear that lead affect gonads directly or through the disruption of reproductive hormones. But it is known that high level of lead decrease the sperm count and motility, including abnormal morphology (Vigeh, et al., 2011). Greenstein, *et al.,* (1987) showed that orchidectomy restored the thymus and raised the total white cell count in 18-month-old rats in which the thymus had virtually disappeared. Similar thymic regeneration was achieved in intact old rats with subcutaneous implants of luteinizing hormone releasing hormone. The activity of the thymus appears to be related to the levels of thymic hormones in the organs. With aging the functional capacity of the thymus may be reduced or not effectively stimulated but it is not completely lost.

Species, strain and sex differences have significant role in the involution of thymus. There is no species in which complete involution takes place, there is always some remnants of thymus at every stage of life (Khosla and Ovalle, 1986). Strain differences can also be important. For example, aged brown Norway female rats thymus contain mostly epithelial cord with less lymphocytes but Wister or WAG strain's female has scarcer epithelial components (Kuper, *et al.,* 1990). According to our study on Swiss mice, as seen in fig. A developing thymus lobules are not well separated and the number of epithelial cell in cortical region is more than medullary region but in case of adult there are numerous epithelial cells in cortical region. There is more space in between the developing epithelial cells in comparison to adult.

5.0 Effects on Developing Thymus:

The increasing awareness of environmental pollution has led to a parallel renewal of interest in metals as potential immunotoxicants. Recently there has been an increased concern about the accumulation of lead in the environment. Lead, today, remains an economically important commodity. It is highly reactive and forms numerous compounds with very different physical and chemical properties, e.g. solubility that affects their bioavailability. One of the major soluble lead compounds is lead acetate trihydrate. It is used as a penetrating agent in cotton dyes, in antifouling paints and insecticides, as a lead coating for metals, as a drier in paints, varnishes, pigment inks, as a colorant in hair dyes and as a processing agent in the cosmetics, perfume and toiletry industries (IARC, 2004). The primary routes of

potential exposure to it are ingestion, inhalation or dermal contact and it is absorbed about 1.5 times faster than any other lead compound.

During development, migration and maturation of T-cell a synchronized set of events occur. The basic needs and required microenvironment for the differentiation and maturation of these cells is provided by primary lymphoid organ. In mice thymus is a primary lymphoid organ where T-cells under goes the maturation and differentiation process, while B-cell maturation and differentiation takes place in red bone marrow. These cells are colonized in thymus and bone marrow around day 13, continuing until birth (Dietert, *et al.,* 2000; Medlock, *et al.,* 1984). After undergoing "thymic education" within thymus, the T-lymphocyte matures into immunocomptent cells, which are then read to encounter foreign antigens. Graham *et al.,* (2011) studies focus that oral exposure of lead in Sprague Dawley female rats alters the organ body weight ratio, most notably of the thymus weight. Lead is a heavy metal and defines as a xenobiotic agent and shows the developmental immunotoxic effects. Adverse effect occurs in offspring's immune system when lead is exposed through full gestation (Bunn, *et al.,* 2001a; Chen, *et al.,* 1999; Faith, *et al.,* 1979; Luster, *et al.,* 1978; Miller, *et al.,* 1998).

Lewin, *et al.,* (1999) reported that Pb levels near four super sites had mean dust Pb levels of 1108mg/kg and soil Pb levels were associated with blood Pb levels in children. In addition to Pb exposure levels, blood-lead levels measured for 2 day at end of exposure in the dams coincided with blood-lead levels (Rojas, *et al.,* 2000; Fischbein, *et al.,* 1993; Sata, *et al.,* 1998). Therefore human exposures to Pb at these levels still exist. Lead acetate is an important immunotoxicant for the phagocytic cells, humoral and cell mediated immunity. The mechanism by which it affects these cells is not understood. (Institoris, *et al.,* 1999; Bunn, *et al.,* 2001; Dietert, *et al.,* 2004; Bishayi & Sengupta, 2006). Its immunosuppressive effect may cause an increased susceptibility of exposed individuals to infection, infestation or the occurrence of tumors.

6.0 Immunotoxicity:

Immune system is affected by any physical chemical or other agents are known as immunotoxicity (Koller, 1987) and the agent by which toxicity caused is known as immunotoxicant. Lead is a well known immunotoxicant which affects the maturation of T-lymphocyte in thymus

at pre and postnatal developmental on low and high levels of exposure. Even low dose of lead exposure at early age critically affects on developing T-cell in thymus (Schuurman, *et al.,* 1994). Immunotoxic reactions commonly immunosuppression (Gopinath, 1996) is defined by selective or general depression in lymphoid organ. In this view several studies show the relation of thymus and lead through which lead decrease the thymus body weight ratio. However high dose of lead exposure decreases food and water consumption and increases stress. Therefore thymus body weight ratio is not a criterion to show the immunosuppression. In some cases a dose-response relationship as well as changes in other lymphoid tissues may be of some help in deciding whether thymic atrophy is a direct effect of immunosuppression or nonspecific response to stress (Greaves, 2000).

The thymus is sensitive to immnotoxicants and show the decreasing size and apoptosis of cortical lymphocytes. A histological change depends upon the dose of immunotoxicant. Following apoptosis of cortical lymphocytes and their removal by macrophages, a decrease in cortical cellularity, loss of the cortex and blurring of normal corticomedullary demarcation are seen. Recent studies show that low levels lead exposure have considerable impact not only on neurological (Verstraeten, *et al.,* 2008) but also on immune system (Dietert and Piepenbrink, 2006; Farrer, *et al.,* 2008). Other studies show that the lead intoxication affects humoral immune activities, function of lymphocyte, cytokine production (McCabe, *et al.,* 2001), diminishes host resistance (McCabe, *et al.,* 1999), cause increased susceptibility to infections, autoimmune diseases and allergy (Bernier, *et al.,* 1995; Colombo, *et al.,* 2004). T-cells are also targeted by lead immunotoxicity (McCabe, *et al.,* 1999; Razani-Boroujerdi, *et al.,* 1999). Therefore we can say that lead contribute in immunosuppression of all animals. Razani-Boroujerdi, *et al* (1999) in vivo studies also show the enhance lymphocyte proliferation activity. Therefore further studies are required for finding the mechanism of lead action by which it affects the animal and its immune system.

Although several mechanisms have been proposed to explain the toxic effects of lead, the exact mechanism is still not clear. One mechanism is that this metal interacts with renal membranes and enzymes and disrupts energy production, calcium metabolism, glucose homeostasis, and ion transport processes (Tsuruoka, *et al.,* 2000).

Another suggested mechanism is by inducing apoptotic cell death as apoptosis induced by lead in several types of cells has been reported, such as in cerebellar neurons (Oberto, *et al.,* 1996) and midbrain dopamine neurons (Tavakoli-Nezhad, *et al.,* 2001). Nevertheless, the exact mechanism of apoptosis induced by lead is not clear and needs to be studied especially in vivo (Zhang, *et al.,* 2004). Teijon, *et al.,* (2010) studies show that oral administration of lead in the form of lead acetate cause intense affects on thymus and a double behavior for the proliferation index. When a control group of animals are compared with lead treated group than proliferation stimulation is observed. So it shows that lead exposure to animal increase the proliferation of thymic lymphocyte.

7.0 Study of Lymphocyte Subpopulations:

Teijon, *et al.,* (2010) work showed that oral exposure of lead cause a double behavior for the proliferation index. During intraperitoneal exposure same effects occur. Therefore both routes of lead exposure induce the T-lymphocyte proliferation. Apoptotic reactions also continue in the thymus if lead is exposed to rats. So, these data corroborate that lead, although it has influence on mechanisms of cell proliferation, does not activate calcium-dependent endonucleases, unlike other environmental pollutants such as dioxins, which activate this enzymatic system (Kaioumova, *et al.,* 2001). It has not been observed that significant necrosis has been generated in thymocytes due to lead. Nevertheless, the structural alterations produced in thymus indicate that it does significantly affect the functionality of this tissue.

The investigation of regulation processes maintaining tissue homeostasis in the thymus showed a significant increase in apoptosis, a decrease in cellular proliferation and a decrease in thymic export as indicated by a reduction in the number of recent thymic emigrants (RTE) in the peripheral blood. The proliferative response of mature thymocytes to Concanavalin A (ConA) was also significantly reduced in socially defeated rats. The strong positive relationship between total thymocyte numbers and peripheral RTE numbers and the long-lasting atrophy of the thymus in defeated animals may suggest negative consequences for the heterogeneity of naïve T cells in the peripheral T cell pool after social stress (Engler and Stefanski, 2003).

8.0 Effects of Vitamin C on Lead Intoxicated Developing Thymus:

The antioxidant might play a role in the treatment of lead poisoning (Gurer, *et al.,* 2001).Vitamins like C and E are capable to control the critical balance of oxidants and antioxidants in the body. Jiao et al., (2010) reported that natural antioxidant treatment reduce the blood lead level by metal ion chelating mechanism. There work also show that addition of vitamin C and natural antioxidant reduce the absorption of lead in lead treated animals. Simon and Hudes (1999) reported that intake of ascorbic acid decrease the blood lead level and control the lead toxicity. Some previous work also reviewed the immune enhancing role of vitamin C (Wintergerst, *et al.,* 2006; Webb, *et al.,* 2007; Ströhle and Hahn, 2009; Thomas and Holt, 1978). Due to its antioxidant properties and collagen synthesis role it contributes in regulation of immune system (Jacob, *et al.,* 1991). So oral intake of vitamin C improve the immune system components such as, leucocytes function, neutrophil and monocyte movement (Lavine *et al*., 1994), natural killer cell activity, lymphocyte proliferation and chemotaxis (Jacob, *et al.,* 1991; Panush, *et al.,* 1982).

Gajawat *et al.,* (2005) studied on prophylactic use of ascorbic acid against radiation and heavy metal intoxication. They reported that prophylactic use of vitamin C is quite effective during lead intoxication and irradiation. By increasing sulphydryl groups and glutathione level of blood, vitamin C protects the biological system.

Vitamin C is effective in protecting against oxidative damage in tissues and also suppresses formation of carcinogens like nitrosamines. There is an inverse relationship with blood pressure and both plasma vitamin C and Vitamin C. Vitamin C has a lowering effect on blood pressure, especially on systolic pressure more than a diastolic pressure. Low levels of plasma vitamin C are associated with stroke and with an increased risk of all cause mortality. Increased consumption of ascorbic acid raises serum ascorbic levels and could decrease the risk of death (Walingo, 2005).

Mega doses of vitamin C increase the levels of antibody that fights against germs and viruses in both stressed and unstressed rats, with greater antibody increase in the unstressed rats (Unknown, 1999). Stressed animals may need larger vitamin C doses for proper function of the immune system. Ali, *et al.,* (2010) noticed that hematological parameters were reduced due to lead acetate-treatment but when Vitamin C was given along with lead the values tend to be normal

at 14 days which suggest that Vitamin C is a good antioxidant is overcome lead toxicity. Furthermore vitamin C was shown to improve the human immune response such as antimicrobicidal natural killer cell activities, lymphocyte proliferation and chemotaxis (Heuser and Vojdani, 1997; Campbell, *et al.,* 1999; Wolf, 1993; pavlovic, *et al.,* 2005; pavlovic and pavlovic, 2005; pavlovic, *et al.,* 2004) indicating the important role of this vitamin in regulating the immune response.

9.0 Conclusions:

* On the basis of above literature we can say that thymus develops gradually from 12th day of gestation to puberty.

* After puberty dramatically retrogressive changes occurs in its size and cellularity. This phenomena known as thymic involution or age associated thymic atrophy. The potential effects of early exposure to lead on thymic development have been not characterized.

* In the above review we tried to evaluate the capacity of lead to alter thymus development in neonatal pups following gestational and lactational exposure.

* The results of various studies suggest that lead can directly influence the development of thymus and functioning of thymocytes. Since, thymulin levels may influence lead induced Immunotoxicity. The embryonic endocrine status may be an important consideration.

* Lead affects on thymus cellularity and maturation process of T-lymphocyte. Many investigations shows that we can treat the lead induced Immunotoxicity by several antioxidants like vitamin C. The immune enhancing role of vitamin C and its detoxification role against free radical treatment are proved by the literature.

* Further studies are required to evaluate the correlation between developing thymus in both sexes and lead exposure during various critical stages of developmental. Along with this the role of antioxidants in abating the effects of lead toxicity on immune system is also important.

References:

1. Agency for toxic Substances and Diseases Registry (1997): Study of Female Former Workers at a Lead Smelter: An Examination of the Possible Association of Lead Exposure with Decreased Bone Density and Other Health Outcome. Atlanta: US Department of Health and Human Services.

2. Ali, F., Singh, K., Rani, S., Ahirwar, V. and Khan, S. (2010): Effect of Ascorbic acid Against Lead (Pb) Toxicity. *IJPSR.,* 1: 81-85.

3. Andrew, H., Brainard, M. D. and Brislen, H. C. (2007): Viewpoint: Learning Professionalism: A view from the Trenches. *Acad. Med.,* 82: 1010-1014.

4. Baldwin, D. R. and Marshall, W. J. (1999): Heavy Metal Poisoning and its Laboratory Investigation. *Ann Clini Biochem.,* 36: 267-300.

5. Banks, W. (1993): Applied Veterinary Histology. Mosby, St. Louis.

6. Bernier, J., Brousseau, P., Krzystyniak, K., Tryphonas, H. and Fournier, M. (1995): Immunotoxicity of Heavy Metals in Relation to Great Lakes. *Environ Health Perspect.,* 103: 23–34.

7. Bishayi, B. and Sengupta, M. (2006): Synergism in Immunotoxicological effects due to Repeated Combined Administration of Arsenic and Lead in Mice. *Int. Immunopharmacol.,* 6: 454 – 464.

8. Bogner, J. *et al.,* (2008): Mitigation of global greenhouse gas emissions from waste: conclusions and strategies from the intergovernmental panel on climate change (IPCC). Fourth Assessment Report. Working Group III (Mitigation). *Was. Manag. Res.,* 26: 11-32.

9. Bruijntjes, J. P., Kuper, C. F., Robinson, J. E., and Schuurman, H. J. (1993): Epithelium-free Area in the Thymic Cortex of Rats. *Dev. Immunol.,* **3**: 113–22.

10. Bunn, T. L., Ladics, G. S., Holsapple, M. P., and Dietert, R. R. (2001a): Developmental Immunotoxicology Assessment in the Rat: Age, Gender and Strain Comparisons after Exposure to Pb. *Toxicol. Methods.,* 11: 41–58.

11. Bunn, T. L., Parsons, P. J., Kao, E. and Dietert, R. R. (2001): Exposure to Lead during Critical Windows of Embryonic Development: Differential Immunotoxic outcome based on Stage of Exposure and Gender. *Toxicol. Sci.,* 64: 57 – 66.

12. Campbell, J. D., Cole, M., Bunditrutavorn, B. and Vella, A. T. (1999): Ascorbic Acid is a Potent Inhibitor of Various forms of T Cell Apoptosis. *Cell. Immunol.,* 194: 1-5.

13. Chen, S., Golemboski, K. A., Sanders, F. S., and Dietert, R. R (1999): Persistent Effect of *in utero* Meso-2, 3-Dimercaptosuccinic Acid (DMSA) on Immune Function and Lead-induced Immunotoxicity. *Toxicol.,* 132: 67–79.

14. Colombo, M., Hamelin, C., Kouassi, E., Fournier, M. and Bernier, J. (2004): Differential Effects of Mercury, Lead, and

Cadmium on IL-2 Production by Jurkat T cells. *Clin. Immunol.,* 111:311–322.

15. Damstra, T. (1977): Toxicological Properties of Lead. *Environ. Health Perspect.,*19: 297-307.

16. De Waal, E. J., Schuurman, H. J., Van Loveren, H. and Vos, J. G. (1997): Differential Effects of 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin, Bis (tri-n-butyltin) Oxide and Cyclosporine on Thymus Histophysiology. *Crit Rev Toxicol.,* 27: 381–430.

17. Dietert, R. R., Etzel, R. A., Chen, D., Halonen, M., Holladay, S. D., Jarabek, A. M., Landreth, K., Peden, D. B., Pinkerton, K., Smialowicz, R. J., and Zoetis, T. (2000): Workshop to Identify Critical Windows of Exposure for Children's Health: Immune and Respiratory Systems Work Group Summary. *Environ. Health Perspect.,* 108: 483–490.

18. Dietert, R. R., Lee, J. E., Hussain, I., Piepenbrink, M. (2004): Developmental Immunotoxicology of Lead. *Toxicol. Appl. Pharmacol.,* 198: 86 – 94.

19. Dietert, R. R. and Piepenbrink, M. S. (2006): Lead and Immune Function. *Crit. Rev. Toxicol.,* 36:359–385.

20. Dijkstra, C., and Sminia, T. (1990): Normal Anatomy, Histology, Immunohistology, Ultrastructure, Rat. In: Hematopoetic System. Monographs on Pathology of Laboratory Animals (T. Jones, J. Ward, U. Mohr, and R. Hunt, eds.), pp. 249–56. Springer-Verlag, Berlin.

21. Elmore, S. A. (2006): Enhanced Histopathology of Thymus. *Toxicol. Pathol.,* 34: 656–665.

22. Engler, H. and Stefanski, V. (2003): Social Stress and T cell Maturation in Male Rats: Transient and Persistent Alterations in Thymic Function. *Psychoneuroendocrinol.,* Vol.: 28: 951-969.

23. Faith, R. E., Luster, M. I. and Kimmel, C. A. (1979): Effect of Chronic Developmental Pb Exposure on Cell-mediated Immune Functions. *Clin. Exp. Immunol.,* 35: 413–420.

24. Farrer, D. G., Hueber, S., Laiosa, M. D., Eckles, K. G., and McCabe, M. J. Jr (2008): Reduction of Myeloid Suppressor Cell Derived Nitric oxide Provides a Mechanistic basis of Lead Enhancement of Alloreactive CD4+T Cell Proliferation. *Toxicol Appl Pharmacol.,* 229:135–145.

25. Fischbein, A., Tsang, P., Luo, J. J., Roboz, J. P., Jiang, J. D., and Bakesi, J. G. (1993): Phenotypic Aberrations of CD31 and CD41 Cells and Functional Impairments of Lymphocytes at Low-level Occupational Exposure to Lead. *Clin. Immunol. Immunopathol.,* 66: 163–168.

26. Gidlow, D. A. (2004): Lead Toxicity In-Depth Review. *Occup. Med.,* 54:76–81.

27. Gojawat, S., Sancheti, G. and Goyal P. K. (2005): Vitamin C against Concomitant exposure to Heavy Metal and Radiation: A study on variation in Hepatic Cellular Counts. *Asian J. Exp. Sci.,* 19: 53-58.

28. Gopinath, C. (1996): Pathology of Toxic Effects on the Immune System. *Inflamm Res*., 45: S74-S78.

29. Graham, D. L. *et al.,* (2011): Effects of Developmental Stress and Lead (Pb) on Corticosterone after chronic and acute Stress, Brain Monoanine and Blood Lead levels in Rats. *Int. J. of Neuroscience.* 29: 45-55.

30. Greaves, P. (2000): Hematopoetic and Lymphatic Systems in Histopathology of Preclinical Toxicity Studies, Interpretation and Relevance in Drug Safety Evaluation, pp. 87–156. Elsevier, Amsterdam.

31. Greenstein, B. D., Fitzpatrick, F. T., Kendall, M. D., and Wheeler, M. J. (1987): Regeneration of the Thymus in old Male Rats Treated with a Stable Analogue of LHRH. *J. Endocr.,* 112**:** 345–350.

32. Grossman, C. J. (1985): Interactions between the Gonadal Steroids and the Immune System. *Sci.,* 227: 257–261.

33. Gurer, H., Ozygunes, H., Saygin, E. and Ercal N. (2001): Antioxidant effect of Taurine against Lead - induced Oxidative Stress. *Arch. Environ. Contam. Toxicol.,* 41: 397- 402.

34. Haley, P. J. (2003): Species Differences in the Structure and Function of the Immune System. *Toxicol.,* 188: 49–71.

35. Haley, P., Perry, R., Ennulat, D., Frame, S., Johnson, C., Lapointe, J. M., Nyska, A., Snyder, P. W., Walker, D. and Walter, G. (2005): STP Position paper: best Practice Guideline for the Routine Pathology Evaluation of the Immune System. *Toxicol. Pathol.,* 33: 404–407.

36. Heuser, G. and Vojdani, A. (1997): Enhancement of Natural Killer Cell Activity and T and B cell Function by Buffered Vitamin C in Patients Exposed to Toxic Chemicals: the role of Protein Kinase-C. *Immunopharmacol. Immunotoxicol.,* 19: 291-312.

37. Hu, H. (1998): Heavy Metal Poisoning. In: Fauci A. S., Braunwald E., Jsselbacher K. J., Wilson J. D., Martin J. B., Kasper D. L., Hauser S. L., Longo D.L. Eds. Harrison's Principles of Internal Medicine, 14 ed., New York: McGraw-Hill, p2564-2569.

38. IARC, International Agency for Research on Cancer - Summaries & Evaluations (2004): Lead and lead compounds: lead and inorganic lead compounds (Group 2b) Organolead

Compounds (Group 3), 10 – 17 February 2004, 87: 230.

39. Institutoris, I., Siroki, O., Desi, I. and Undeger, U. (1999): Immunotoxicological Examination of Repeated Dose Combined Exposure by Dimethoate and two Heavy Metals in Rats. *Hum. Exp. Toxicol.,* 18: 88–94.

40. Jacob, A. J. *et al.,* (1991): Immunocompetence and Oxidant Defense during Ascorbate Depletion of Healthy Men. *Am. J. Clin. Nutr.,* 1991. 54: 1302S – 1309S.

41. Jiao, J., Lu, G., Liu, X. Zhu, H. and Zhang, Y. (2010): Reduction of Blood Lead levels in Lead-Exposed Mice by Dietary supplements and Natural antioxidants. *J. Sci. Food Agri.,* 91: 485-491.

42. Kaioumova, D., Kaioumov, F., Opelz, G. and Süsal, C. (2001): Toxic Effects of the Herbicide 2, 4- Dichlorophenoxyacetic Acid on Lymphoid Organs of the Rat. *Chemosphere.* 43: 801–805.

43. Khosla, S. and Ovalle, W. K. (1986): Morphology and Distribution of Cystic Cavities in the Normal Murine Thymus. *Cell Tiss. Res.,* 246: 531–542.

44. Kilikdar, D. *et al.,* (2011): Protective Effect of Aqueous Garlic Extract against Lead induced Hepatic injury in Rats. *Ind. J. Exper. Bio.,* 49: 498-510.

45. Kimmel, C. A., Grant, L. D. and Sloan, C. S. (1976): Chronic Lead Exposure: Assessment of Developmental Toxicity. *Abstr. Teratol. Soc.,* Carmel,

46. Koller, L. D. (1987): Immunotoxicology Today. *Toxicol. Pathol.,* 15: 346–351.

47. Kuper, C. F., Beems, R. B. and Hollanders, M. H. (1990): Development and Aging, Thymus, Rat. In Hematopoietic System, Monographs on Pathology of Laboratory Animals, T. C. Jones, J. M.Ward, U. Mohr, and R. D. Hunt, (eds.), pp. 257–63. Springer-Verlag, Berlin.

48. Kuper, C. F., Beems, R. B., Bruijntjes, J. P., Schuurman, H. J. and Vos, J. G. (1992): Normal Development, Growth, and Aging of the Thymus. In Pathobiology of the Aging Rat. Volume 1. U. Mohr, D. L. Dungworth, and C.C. Capen, (eds.), pp. 25–48. ILSI Press, Washington, DC.

49. Kuper, C. F., de Heer, E.,Van Loveren, H. and Vos, J. G. (2002): Immune System. In Handbook of Toxicologic Pathology, Volume 2. W. M. Haschek, C. G. Rousseaux, and M. A. Wallig, (eds.), pp. 585–644. Academic Press, San Diego.

50. Kuper, F., Schuurman, H. J. and Vos, J. G. (1995): Pathology in Immunotoxicology. In: Methods in Immunotoxicology (G. Burleson, J.

Dean, and A. Munson, eds.), Vol. 1, pp. 397–436. Wiley-Liss, New York.

51. Lane, R. E. (1949): The Care of the Lead Worker. *Br. J. Ind. Med.,* 6: 125–143.

52. Levin, S. M., Goldberg, M. and Doucette, J. T. (1997): The Effects of OSHA Lead Exposure in Construction Standard on Blood Lead levels among Iron workers Employed in Bridge Rehabilitation. *Am. J. Ind. Med.,* 31: 303–309.

53. Levine, M. *et al.,* (1994): Ascorbic Acid in Neutrophils. In: Natural Antioxidants in Health and Disease (Frei B, ed). San Diego: Academic Press, 1994; pp 469 – 488.

54. Lewin, M. D., Sarasua, S. and Jones, P. A. (1999): A Multivariate linear Regression Model for Predicting Children's Blood Lead levels based on Soil Lead levels: A Study at four Superfund Sites. *Environ. Res.,* 81, 52–61.

55. Luster, M. I., Faith, R. E. and Kimmel, C. A. (1978): Depression of Humoral Immunity in Rats Following Chronic Developmental Pb Exposure. *J. Environ. Pathol. Toxicol.,* 1, 397–402.

56. McCabe, M. J. Jr. Singh, K. P. and Reiners, J. J. Jr. (1999): Lead Intoxication Impairs the Generation of a Delayed type Hypersensitivity Response. *Toxicol.,* 139: 255–264.

57. McCabe, M. J. Jr., Singh, K. P. and Reiners, J. J. Jr. (2001): Low Level Lead Exposure in Vitro Stimulates the Proliferation and Expansion of Alloantigen-reactive CD4 (high) T Cells. *Toxicol. Appl. Pharmacol.,* 177: 219–231.

58. Medlock, E. S., Landreth, K. S. and Kincade, P. W. (1984): Putative B Lymphocyte Lineage Precursor Cells in early Murine Embryos. *Dev. Comp. Immunol.,* 8**:** 887–894.

59. Miller, T. E., Golemboski, K. A., Ha, H. A., Bunn, T., Sanders, F. S. and Dietert, R. R. (1998): Developmental Exposure to Pb causes Persistent Immunotoxicity in Fischer 344 Rats. *Toxicol. Sci.,* 42: 129–135.

60. Oberto, A., Marks, N., Evans, H. L. and Guidotti, A. (1996): Lead Promotes Apoptosis in Newborn Rat Cerebellar Neurons: pathological implications. *J. Pharmacol. Exp. Ther.,* 279: 435–442.

61. Panush, R. S., Delafuente, J. C., Katz, P. and Johnsin, J. (1982): Modulation of certain Immunologic Responses by Vitamin C. III. Potentiation of *In Vitro* and *In Vivo* Lymphocyte responses. *Int. J. Vitam. Nutr. Res.,* 23: 35–47.

62. Patrick, L. (2006): Lead toxicity, a review of the literature. Part I: exposure, evaluation, and treatment. *Altern. Med. Rev.,* 11: 2–22.

63. Pavlovic, V., Cekie, S., Rankovic, G. and Stojikovic, N. (2005): Antioxidant and Pro-

oxidant effect of Ascorbic acid. *Acta. Med. Midianae.,* 44: 65-68.

64. Pavlovic, Z. and Pavlovic, V. (2005): The Effect of Ascorbic acid on Pathohistological Tumer Characteristics and Phenotype Characteristics of Lymphocyte during the Development of Experimental Mammary Carcinoma in Mice. *Acta. Med. Midianae.,* 44: 23-31.

65. Pavlovic, Z. *et al.,* (2004): The Effect of Ascorbic Acid on Development of Experimental Mammary Carcinoma in Mice. *Acta. Med. Medianae.*, 43: 5-9.

66. Pearse, G. (2006): Normal Structure, Function, and Histology of the Thymus. *Toxicol. Pathol.,* 34: 504–514.

67. Pizzol, M., Thomsen, M. and Anderson, M. S. (2010): Long-term Human Exposure to Lead from Different Media and intake Pathways. *Sci. Total Environ.,* 408: 5478-5488.

68. Razani-Boroujerdi, S., Edwards, B. and Sopori, M. L. (1999): Lead Stimulates Lymphocyte Proliferation through Enhanced T cell B Cell Interaction. *J. Pharmacol. Exp. Ther.,* 288: 714–719.

69. Reiter, L. W., Anderson, G. E., Laskey, J. W. and Cahill D. F. (1975): Development and Behavioral Changes in the Rat during Chronic Exposure to Lead. *Environ. Health Perspect.,* 12: 119-123.

70. Rojas, M., Squillante, G., Medina, E., de Rojas, O. and Sarmiento, A. (2000): Environmental Factors Associated with Blood Lead Levels in Venezuelan Children. *Vet. Hum. Toxicol.,* 42: 174–177.

71. Sata, F., Araki, S., Tanigawa, T., Morita, Y., Sakurai, S., Nakata, A. and Katsuno, N. (1998): Changes in T-cell Subpopulations in Lead Workers. *Environ. Res.,* 76, 61–64.

72. Schuurman, H. J., Kuper, C. F. and Vos, J. G. (1994): Histopathology of the Immune System as a Tool to assess Immunotoxicity. *Toxicolo.,* 86, 187– 212.

73. Sharma, V., Kansal, L., Sharma, A., Lodi, S. and Sharma, S. H. (2011): Ameliorating effect of *Coriandrum Sativum* Extracts on Hematological and Immunological variable in an Animal Model of Lead Intoxication. *J. of Pharm. and Allied Health Sci.,*

74. Simon, J. A. and Hudes E. S. (1999): Relationship of Ascorbic acid to Blood leads levels. *JAMA* 281: 2289-2293.

75. Smith, J. D. M., Mielke, H. W. and Heneghan J.B. (2008): Sub chronic Lead Feeding Study in Male Rats. *Arch. Environ. Contam. Toxicol.*, 55: 518-528.

76. Smith, P. F. and Carnus, J. M. (2008): The Sustainable Management and Protection of Forests Analysis of the Current Position Globally. *Ambio.* 37: 254-262.

77. Strohle, A. and Hahn, A. (2009): Vitamin C and Immune Function. *Med. Monatsschr. Pharm.,* 32: 49 – 54.

78. Suster, S. and Rosai, J. (1992): Thymus. In Histology for Pathologists (S. Sternberg, ed.), pp. 261–75. Raven Press, New York.

79. Tavakoli-Nezhad, M., Barron, A. J. and Pitts, D. K. (2001): Postnatal Inorganic Lead Exposure Decreases the Number of Spontaneously Active Midbrain Dopamine Neurons in the Rat. *Neurotoxicol.,* 22: 259–269.

80. Teijón, C., Blanco, M. D., Romero, C. S., Beneit, J. V., Villarino, A. L., Guerrero, S. and Olmo, R. (2010): Study of Response of Thymic and Submaxillary Lymph Node Lymphocytes to Administration of Lead by Different Routes. *Biol. Trace Elem. Res.,* 135: 74–85.

81. Terszowski, G. *et al.,* (2006): Evidence for a Functional Second Thymus in Mice. *Sci.,* 312: 284–7.

82. Thomas, W. R. and Holt, P. G. (1978): Vitamin C and Immunity: An Assessment of the Evidence. *Clin. Exp. Immunol.,* 32: 370 – 379.

83. Timbrell, J. A. (1995): Introduction to Toxicology. 2nd ed., London : Taylor and Francis

84. Tryphonas, H. *et al.,* (2004): Oral (gavage), In Utero and Post-natal Exposure of Sprague–Dawley Rats to Low Doses of Tributyltin Chloride. Part II: Effects on the Immune System. *Food Chem. Toxicol.,* 42, 211–220.

85. Tsuruoka, S., Sugimoto, K., Muto, S., Nomiyama, K., Fujimura, A. and Imai, M. (2000): Acute Effect of Cadmium-metallothionein on Glucose and Amino Acid Transport across the Apical Membrane of the Rabbit Proximal Tubule Perfused *In Vitro*. *J. Pharmacol. Exp. Ther.,* 292: 769–777.

86. Unknown, T. (1999): New Orleans (AP).

87. Verstraeten, S. V., Aimo, L. and Oteiza, P. I. (2008): Aluminium and Lead: Molecular Mechanisms of Brain Toxicity. *Arch. Toxicol.,* 82: 789–802.

88. Vigeh, M., Smith, D. R. and Hsu, P. C. (2011): How does Lead induce Male Infertility. *Iran. J. of repro. Med.,* 9: 1-8.

89. Walingo, K. M. (2005): Role of Vitamin C (Ascorbic Acid) on Human Health- a review. *AJFAND.,* 5.

90. Webb, A. L. and Villamor, E. (2007): Update: Effects of Antioxidant and Non-antioxidant Vitamin Supplementation on Immune Function. *Nutr. Rev.,* 65: 181–217.

91. Williams, F., Robertson, R. and Rowocth, M. (1999): Scottish Centre for Infection and Environmental Health. Ist edn. Glasgow: SCEIH.

92. Winder, C. (1993): Lead, Reproduction and Development. *Neurotoxicol.,* 14: 303-317.

93. Wintergerst, E. S., Maggini, S. and Hornig, D. H. (2006): Immune-enhancing Role of Vitamin C and Zinc and Effect on Clinical Conditions. *Ann. Nutr. Metab.,* 50: 85–94.

94. Wolf, G. (1993): Uptake of Ascorbic Acid by Human Neutrophil. *Nutr. Rev.,* 51: 337-338.

95. Zhang, J., Wang, X. F., Lu, Z. B., Liu, N. Q. and Zhao, B. L. (2004): The Effects of Meso-2, 3-Dimercaptosuccinic Acid and Oligomeric Procyanidins on Scute Lead Neurotoxicity in Rat Hippocampus. *Free Radic. Biol. Med.,* 37: 1037–1050.