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Ultrastructural Alterations of Peripheral Blood Lymphocytes of White Rats (*Rattus norvegicus*) Exposed to Lead

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Abstract: The present study was designed to investigate the ultrastructural alterations of the peripheral blood lymphocytes of rats exposed to lead. For this purpose, a group of 10 male Wistar albino rats received lead as 0.25% lead acetate trihydrate in drinking water for six months. Blood samples were collected from all experimental animals to separate buffy coats which were processed for electron microscopy. Lymphocytes of the exposed animals revealed obvious fine changes which included nuclear and organellar changes. The lymphocytes nuclei showed deep invaginations of the nuclear envelope and marked chromatin condensation. Mitochondria disclosed swelling and deterioration of their cristae. Rough Endoplasmic Reticulum (RER) was markedly dilated and proliferated in considerable number of lymphocytes. It is concluded that lead can induce significant ultrastructural changes in the peripheral blood lymphocytes. The demonstrated changes may adversely affect the lymphocytes functions which are greatly concerned with the immune status of the exposed subjects.

Key words: Lead, toxicity, rats, lymphocytes, ultrastructure

Introduction

Lead, the most ubiquitous toxic metal, is one of the hazardous enviroumental pollutants and is probably the most common form of insidious metallic poisons encountered in all biologic systems (UNEP, 1984; Shy, 1990; Goyer, 1991; Jones, 1991; Fishbein, 1992; Schulte *et al*., 1994). The toxic effects of lead extend to many tissues such as heamatopoietic, hepatic, renal, lymphoid, nervous and reproductive tissues (Goyer, 1991; Dean and Murray, 1991; Jarrar, 2003; Taib *et al*., 2004).

Concerning the effects of lead on haemopoiesis, chronic lead poisoning causes anemia by interfering with erythropoiesis, accelerating red blood cells destruction, i.e., reducing erythrocyte life span and suppressing hemoglobin synthesis (ATSDR, 1992; McDowell, 1992). Also, it induces neutrophilia, eosinophilia, lymphocytosis and monocytosis (Tchernitchin *et al*., 1997). Moreover, lead toxicity results in defects of neutrophil functions, including the bactericidal activity (Governa *et al*., 1989; Queiroz *et al*., 1993; Hrycek and Kalina, 1996; Basaran and Undeger, 2000; Mushtakova *et al*., 2005).

Relatively limited ultrastructural investigations have been done on leucocytes of the experimental animals exposed to lead (Ghachirove and Gracheva, 1975; Sliwa-Tomczok and Tomczok, 1990). Studies on the alterations of leucocytes at the ultrastructural level due to chronic lead intoxication are rather limited and have not yet been indentified. Therefore, the present study was intended to elucidate the ultrastructural alterations of lymphocytes of rats chronically exposed to lead.

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Materials and Methods

Experimental Animals

A total of 20 male Wistar albino rats (*Rattus norvegicus*) of the same age weighing 110-130 g, of the King Saud University colony, were used.

Experimental Design

Animals were randomly divided into two groups of 10 rats each and maintained under the standard laboratory conditions including ambient temperature (23-24°C). The animals receieved water and food *ad libitum*. Following a period of acclimatization (7 days), lead acetate trihydrate was administered via drinking water at the concentration of 0.25% for the exposed group, while sodium acetate replaced lead acetate for the control group. The experimentation period extended for six months. At the end of this period animals were sacrificed and blood was collected from all experimental animals into tubes containing a proper anti-coagulant (ethylenediamine tetracetic acid, EDTA).

Electron Microscopy

The anti-coagulated blood samples were mixed with equal volume of 3% glutaraldehyde in sodium cacodylate buffer (pH 7.2) and kept for 6 h at 4°C. The blood samples were then centrifuged at 3000 rpm for 20 min to separate buffy coats which were then placed in the same fixative for additioual 4 h. The buffy coats were subsequently dehydrated through ascending grades of ethanol, cleared in propylene oxide and finally embedded in epoxy resin (Epon: Araldite mixture). Ultrathin sections from buffy coats (70-80 um) were made on an ultra-microtome (Leica UCT, Austria) using a diamond knife, mounted on copper grids and contrasted with uranyl acetate and lead citrate. The contrasted ultrathin sections were observed under a transmission electron microscope (JEOL, TEM 1011) operating at 80 kV.

Results

Lymphocytes of the Control Animals

Most of peripheral blood lymphocytes of the control animals are the small-sized ones which are round cells having a narrow rim of cytoplasm surrounding a non-lobulated nucleus (Fig. 1). The cytoplasmic contents are scarce and largely involve free ribosomes and tiny rudimentary Golgi complex. There are few dense azurophil granules (lysosomes) and clear vacuoles, but there are no specific granules. Enoplasmic Reticulum (ER) in not well-developed and only few profiles of RER, scattered polyribosomes and reiltively large number of free ribosomes are recognizable. Mitochondria are very few in number. The round nucleus of lymphocytes is often slightly indented, nuclear chromatin is packed at the nuclear periphery and nucleoli are not present (Fig. 2). Plasma membrane has a few pseudopodia. Fewer number of medium-sized lymphocytes are also seen and these cells are identified by their relatively larger nuclei and the more abundant cytoplasmic mass.

Lymphocytes of the Exposed Animals

Lymphocytes in peripheral blood of the exposed animals were the predominant leucocytes, beside neutrophils, observed in the examined ultr-αthin sections. Many lymphocytes revealed nuclear abnormalities, the most common was the various degrees of invagination of the nuclear envelope. The nuclear invagination, in considerable number of lymphocytes, appeared as macroclefts which were deep enough to nearly divide the altered nuclei into two or more portions (Fig. 3 and 4). The resultant nuclear segments were connected by a narrow bridge (s) of chromatin. Widening of the space between the outer and inner nuclear membranes was frequent in the cleaved nuclei showing deep invaginations.

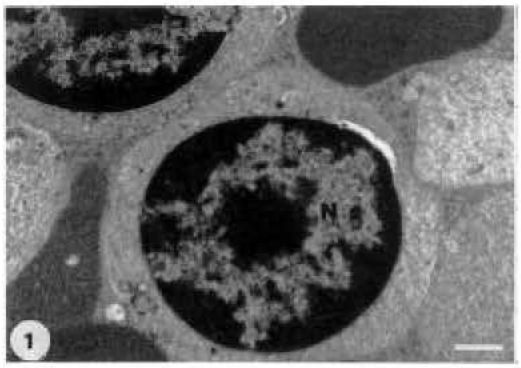

Fig. 1: Lymphocyte of a control rat showing a round nucleus (N) surrounded by a narrow rim of cytoplasm. Scale bar = 1 μm

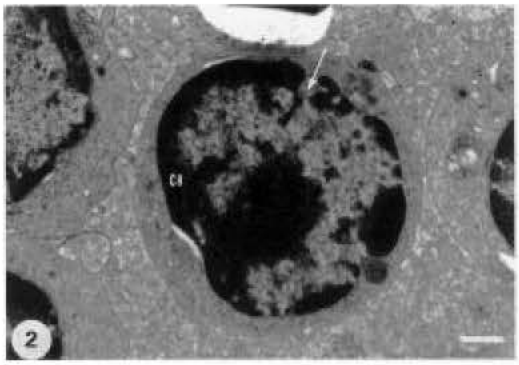

Fig. 2: Lymphocyte of a control rat showing slight nuclear indentation (arrow) and peripheral clumping of the nuclear chromatin (CH). Scale bar = 1 μm

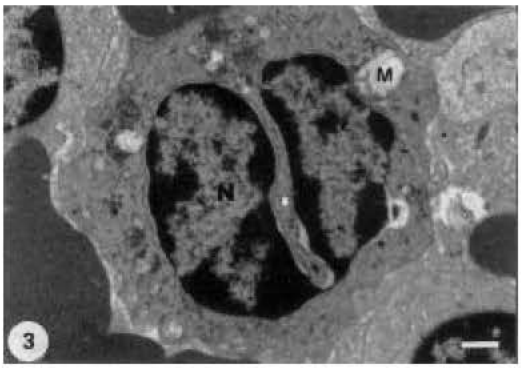

Fig. 3: Lymphocyte of an exposed rat showing deep invagination (*) of the nucleus (N) which is nearly divided into two portions. Mitochondria (M) are swollen and have deteriorated cristae. Scale bar = 1 μm

Fig. 4: Lymphocyte of an exposed rat showing segmentation of the nucleus (N) due to several deep invaginations. Mitochondria (M) are swollen. Scale bar = 1 μm

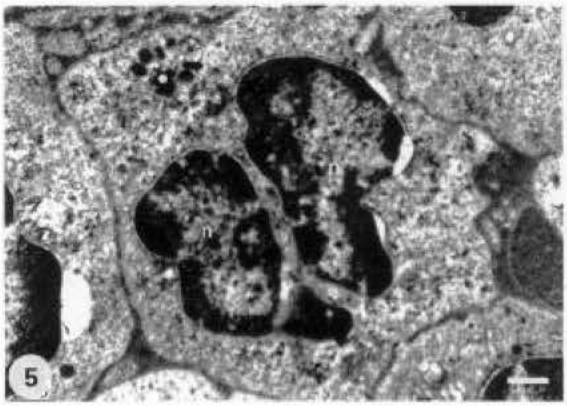

Fig. 5: Complete separation of the nuclear portions (n) resulting from deep invaginations in a lymphocyte nucleus of an exposed rat. Note the collection of the azurophil granules (*). Scale bar = 1 μm

Mitochondria of the altered cells were swollen and had disoriented or deteriorated cristae. Cytoplasm of these cells contained varied sized vesicles and increased number of azurophil granules. Some lymphocytes showed obvious separation of the nuclear portions resulting from the deep invaginations (macroclefts) which completely cleaved the altered nuclei (Fig. 5). The separated segments were of irregular size and shape and some had obviously condensed chromatin (Fig. 6). Another form of nuclear abnormalities was the marked irregularity of the nuclear envelope which gave the appearance of nuclear protrusions (Fig. 7). Occasionally, the irregular nuclei showed intranuclear cytoplasmic invaginations which were represented by moderately dense bodies delimited by a single membrane and surrounded by a narrow empty space (Fig. 8). These bodies probably represented intranuclear pocket-like structures or pseudo-inclusions. Chromatin condensation was also a form of nuclear abnormalities, the highly condensed chromatin was the heterochromatin with less contribution for euchromatin (Fig. 9). Markedly shrunken nuclei with highly condensed chromatin were seen in some lymphocytes (Fig. 10). Noticeably proliferated rough endoplasmic reticulum was recognized in some lymphocytes which had shrunken nuclei (Fig. 11), as well as in lymphocytes with nearly regular nuclei (Fig. 12). The fine granular content in cisternae of the proliferated RER was revealed on higher magnification of the alterted lymphocytes (Fig. 13).

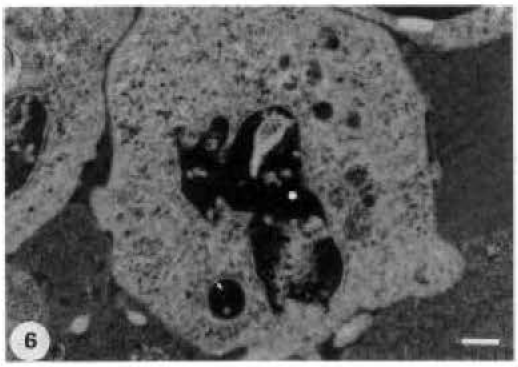

Fig. 6: A nuclear portion (*) in a lymphocyte of an exposed rat. Note the marked condensation of the nuclear chromatin, and the large azurophil granule (arrow). Scale bar = 1 μm

Fig. 7: Nucleus (N) of a lymphocyte of an exposed rat showing obvious protrusions of the nuclear envelope. Note the site of the deteriorated organelles (*). Scale bar = 1 μm

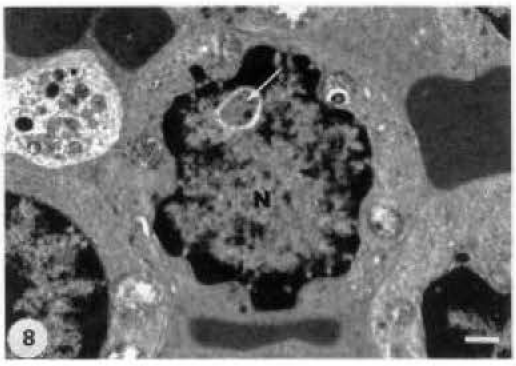

Fig. 8: Irregular nucleus (N) showing protrusions and moderately dense intranuclear body (arrow) which possibly represents a process of cytoplasmic intranuclear invagination. Scale bar = 1 μm

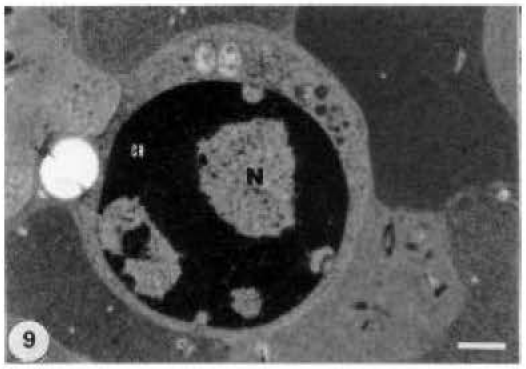

Fig. 9: Nucleus (N) of a lymphocyte of an exposed rat showing highly condensed chromatin (CH). Scale bar = 1 μm

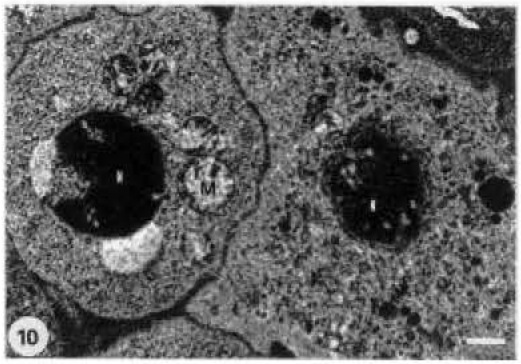

Fig. 10: Lymphocytes of an exposed rat showing obviously shrunken nuclei (N) which have markedly condensed chromatin. Mitochondria (M) are deteriorated. Scale bar = 1 μm

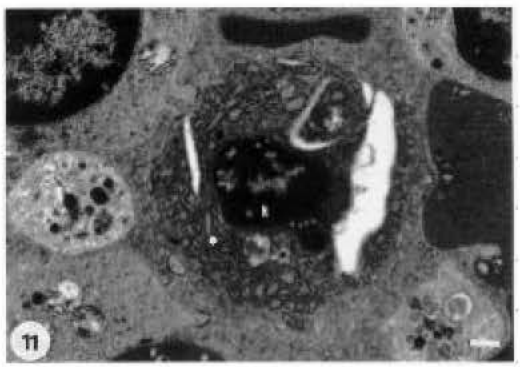

Fig. 11: Lymphocyte of an exposed rat showing extensively proliferated RER (*). Note the markedly shrunken nucleus (N) which possesses condensed chromatin. Scale bar = 1 μm

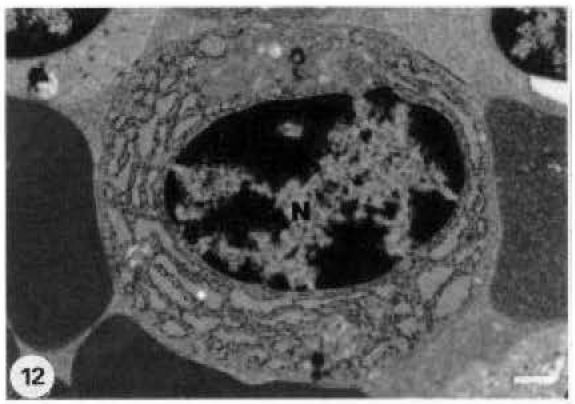

Fig. 12: Lymphocyte of an exposed rat showing proliferated and dilated RER (*). Nucleus (N) is relatively compressed. Scale bar = 1 μm

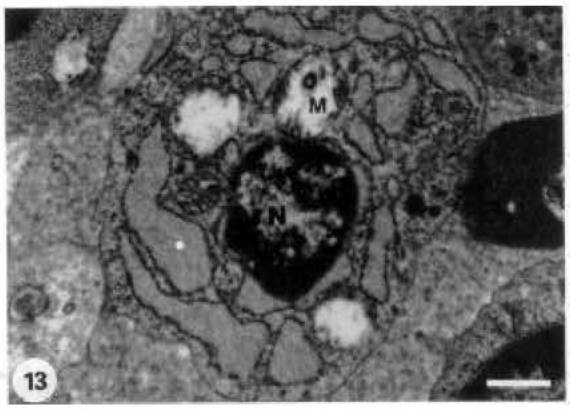

Fig. 13: Higher magnification of a lymphocyte of an exposed rat showing markedly dilated RER (*) which contains fine granular material. Nucleus (N) is shrunken and mitochondria (M) are deteriorated. Scale bar = 1 μm

Discussion

Most of the peripheral blood lymphocytes examined in the present study were the small-sized ones. Although the peripheral blood lymphocytes have uniform appearance, but they involve different populations (Bloom and Fawcett, 1975). The majority of these cells are recirculating long-lived ones which participate in cell-mediated immunity. The noticed small-sized lymphocytes has a little cytoplasmic mass, in this respect the activity state of lymphocytes affects the amount of cytoplasm, being larger in medmium- and large-sized lymphocytes (Wheater *et al*., 1987).

In a previous study (Taib *et al*., 2004). Comparable mitochondrial changes to that observed here, have been described in hepatocytes of lead-intoxicated rats. Mitochondria is one of the main target organelles in lead toxicity (Aldridge, 1970; Racker, 1970). The present results support the conclusion that mitochondria are highly susceptible to lead toxicity. The observed mitochondrial swelling is probably ascribed to osmolarity changes, which eventually lead to influx of ions and subsequently fluid accumulation with ultimate mitochondrial swelling (King *et al*., 1983). Lead may also interact with mitochondrial membranes and thus results in impaired electron transport which accentuates the

mitochondrial swelling. Mitochondrial swelling has been also described in tissues such as heart, cerebellum and kidney of lead exposed animals (Bull, 1980; Jarrar, 2001). Mitochondria with noticeable membrane changes exhibit compromised oxidative phosphorylation and reduced ATP production, since these processes are largely dependant on the integrity of mitochondrial cristae (Kendall and Scanlon, 1985; Oskarsson *et al*., 1992).

Presently, considerable number of lymphocytes revealing marked RER proliferation and dilatation were detected in the lead intoxicated animals. It has been stated that lymphocytes with extensive endoplasmic reticulum are cells concerned with protein synthesis (Bloom and Fawcett, 1975). However, these cells which possessed proliferating RER are presumably highly dividing cells, since chronic lead toxicosis has been found to induce lymphocytosis (Basaran and Undeger, 2000). Many peripheral lymphocytes of the currently intoxicated rats disclosed remarkable nuclear chromatin condensation, which has been described as an early morphological apoptotic change (Cohen and Duke, 1984; English *et al*., 1989). However, to confirm the ability of lead to provoke apoptotic changes in the circulating lymphocytes, further studies at the molecular level should be conducted.

The increased number of azurophil granules, possibly primary lysosomes, in the currently altered lymphocytes is presumably an indication for enhanced autophagic activity related to the presence of degenerated or deteriorated mitochondria. In this case, autophagic activity is considered a removal mechanism of the defective organelles in the affected cells.

In general, the interpretation of lead-induced toxicity of lymphoid cells is based on the fact that lead is a sulfhydryl alkylating agent with a high affinity for subcellular sulfhydryl group (Goyer, 1991). Thus, lead is able to associate with the membrane and intracellular thiols which are important in lymphocyte activation, proliferation and differentiation.

Lymphocytes are known to play a central role in all immunological defensive mechanisms (Bloom and Fawcett, 1975; Wheater *et al*., 1987). The presently demonstrated ultrastructural changes of lymphocytes of exposed rats undoubtedly affect this role and thus the immune status can be significantly compromised. In this respect, lead is one of the heavy metals that exert immunotoxic effects and it has the ability to impair both cell-mediated immunity and antibody-mediated host resistance (Dean and Murray, 1991). It has been found that chronic lead exposure suppresses cell-mediated immunity parameters including delayed type hypersensitivity and lymphoproliferation in response to mitogens (Faith *et al*., 1979; Blakely and Archer, 1982). The lead-induced immunosuppression would increase the host susceptibility to pathogens.

Conclusively, the present results indicate that chronic lead exposure can induce subcellular changes in peripheral blood lymphocytes of the exposed animals. Further studies should focus on the mechanism (s) through which lead could provoke such changes using the recent molecular tools.

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References

Agency for Toxic Substances and Disease Registry (ATSDR), 1992. The nature of lead poisoning in children in the united states: A Report to Congress. Atlanta.

Aldridge, W.N., 1970. Effect of Metals on Cells, Subcellular Elements and Macromelecules. Maniloff, J., J.R. Coleman and M.W. Miller (Eds.). Thomas and Springfield, Illinios, pp: 255-277.

Basaran, N. and U. Undeger, 2000. Effects of lead on immune parameters in occupationally exposed workers. Am. J. Med., 38: 349-354.

Blakely, B.R. and D.L. Archer, 1982. Mitogen stimulation of lymphocytes exposed to lead. Toxicol. Applied Pharmacol., 62: 183-189.

Bloom, W. and D.W. Fawcett, 1975. A Textbook of Histology. 10th Edn. W.B. Saunders Co., Philadelphia, London.

Bull, R.J., 1980. Lead and Energy Metabolism. In: Lead Toxicity. Singhal, R.L. and J.A. Thomas (Eds.), Urban and Schwarzenberg, Munich.

Cohen, J.J. and R.C. Duke, 1984. Glucocotricoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. J. Immunol., 132: 38-42.

Dean, J.H. and M.J. Murray, 1991. Toxic Responses of the Immune System. In: Toxicology: The Basic Science of Poisons. Amdur, M.O., J. Doull and C.D. Klaassen (Eds.). 4th Edn. Pergamon Press, New York, pp: 282-333.

English, H.F., N. Kyprianou and J.T. Isaacs, 1989. Relationship between DNA fragmentation and apoptosis in the programmed cell death in the rat prostate following castration. Prostate, 15: 233-250.

Faith, R.E., M.I. Luster and C.A. Kimmel, 1979. Effect of chronic lead exposure on cell mediated immune function. Clin. Exp. Immunol., 35: 413-424.

Fishbein, A., 1992. Lead poisoning: 1-Some clinical and toxicological observations of the effects of occuptinal lead exposure among firearms instructors. Israel J. Med. Sci., 28: 560-572.

Ghachirove, D.G. and A.I. Gracheva, 1975. Ultrastructure of the cells of the peripheral blood in experimental lead poisoning. Farmako Toksikol., 38: 465-467, 17: 16-21.

Governa, M., I. Viscosa, M. Valentino and I. Maarchiseppe, 1989. Lead interference in actin redistribution induced chemotactic peptide in human neutrophils. ATLA.

Goyer, R.A., 1991. Toxic Effects of Metals. In: Toxicology: The Basic Science of Poisons. Amdur M.O. Doull, J. and C.D. Klaassen (Eds.). 4th Edn. Pergamon Press, New York, pp: 623-680.

Hrycek, A. and M. Kalina, 1996. The influence of lead on neutrophils. Wiad Lek., 49: 103-106.

Jarrar, B.M., 2001. Ultrastructural alterations in proximal tubule cells induced by lead. Pak. J. Biol. Sci., 4: 1281-2001.

Jarrar, B.M., 2003. Histological and histochemical alterations in the kidney induced by lead. Ann. Saud. Med., 23: 10-15.

Jones, M.M., 1991. New developments in therapeutic chelating agents as antidotes for metal poisoning. Toxicology, 21: 209-233.

Kendall, R.J. and P.F. Scanlon, 1985. Histology and ultrastructure of kidney tissue from ringed turtle doves that ingested lead. J. Environ. Pathol. Toxicol. Oncol., 6: 85-96.

King, D.W., C.M. Fenoglio and J.H. Leckowich, 1983. General Pathology. Principles and Dynamics. Lea and Febiger, Philadelphia.

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press (San Diego).

Mushtakova, V., V. Fomina and V. Rgovin, 2005. Toxic effect of heavy metals on human blood neutrophils. Biol. Bull. Russ. Acad. Sci., 32: 276-278.

Oskarsson, A., L. Jorhem, Sundberg, J.G. Nilsson and L. Albanus, 1992. Lead poisoning in cattle transfer of lead to milk. Sci. Total. Environ., 111: 83-94.

Queiroz, M.S., M. Almeida, M.L. Gallo and N.F. Hoehr, 1993. Defective neutrophil function in workers occupationally exposed to lead. Pharm. Toxicol., 72:73-77.

Racker, E., 1970. Membranes of Mitochondria and Chloroplasts. Van Nostrand-Reinhold Press, New York.

Schlute, S., W.E. Muler and K.D. Frieberg, 1994. *In vivo* exposure to lead does not influence muscarinic receptors in the frontal cortex of the mouse brain. Toxicology, 93: 99-112.

Shy, C.M., 1990. Lead in petrol: The mistake of the XXth century. Wld. Hlth. Statis., 43: 168-175.

Sliwa-Tomczok, W. and J. Tomczok, 1990. Electron microscopical localization of the lead peripheral blood neutrophils of the rat with timm sulphide silver method and X-ray probe microanalysis. Mikrosk. Anat. Forsch., 104: 458-464.

Taib, N.T., B.M. Jarrar and M. Mubarak, 2004. Ultrastructural alterations in hepatic tissues of white rats (*Rattus norvegicus*) induced by lead experimental toxicity. Saudi J. Biol. Sci., 11: 11-20.

Tchernitchin, A.N., R. Villagra and N.N. Tchernitchin, 1997. Effect of chronic exposure to lead on immature rat leucocytes. Med. Sci. Res., 25: 355-357.

UNEP., 1984. List of environmental dangerous chemical substances of global significance. International Register for Potentially Toxic Chemical, United Nations Environmental Programme.

Wheater, P.R., H.G. Burkitt and V.G. Daniels, 1987. Functional Histology. 2nd Edn. Churchill Livingstone, Edinburgh, London.