Attenuation of Pb toxicity by virgin olive oil and vitamin C

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Article published on October 30, 2017

Key words: Pb, Rat, Vitamin C, Olive Oil, Immunoglobulins

Abstract
The present work was devoted to study the protective antioxidant role of vitamin C and Olive Oil in Wister rats fed a Pb contaminated diet. Females were given, either Pb, Pb-vitamin C (Pb-Vit C) in drinking water or Pb-Olive Oil (Pb-OO) in their diet for 4 weeks. Serum immunoglobulins (Igs), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), total cholesterol, triglycerides, calcium and iron, in addition to haematological parameters were evaluated. During four weeks trial, the most important results showed a significant increase in Igs, LDL, White Blood Cells (WBC), Lymphocytes, with a significant decrease in HDL of Pb group. However, the Pb-Vit C and Pb-OO groups showed a significant inverse finding, by an increase in HDL, total cholesterol, and a decrease in Igs. Concerning WBC counts, significant differences were found between the two combined groups when compared to the control. The concentration of triglycerides was decreased significantly in Pb-Vit C group compared to the control. The level of calcium was not affected in all groups, but those of iron, red blood cells (RBC) and haemoglobin (Hb) were statistically altered in all treated groups when compared to control. To conclude, supplementation of vitamin C and olive oil to rats fed a Pb contaminated diet has remarkably modulated the immune system, reduced the levels of LDL and the triglycerides, which perhaps was due to a decrease of the metal intestinal absorption and tissue accumulation.

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Introduction

Lead is being a global environmental contaminant due to its significant role in modern industry. However, both occupational and environmental exposure remains a serious problem in many developing and industrializing countries. It has many undesired effects, including neurological, behavioural, immunological, renal, hepatic, and haematological and biochemical dysfunctions. (Flora et al., 2003).

Absorbed lead following oral ingestion is carried via blood to soft tissues where 95 per cent of blood Pb is transported on the erythrocytes as blood diphosphate; this might be the reason of increasing Pb concentration in the blood following oral exposure (Livertoux, 2000).

Moreover, an association between atherosclerosis and lead exposure is biological plausible, in which microscopic analysis of lead intoxicated animals has indicated fatty degeneration of the myocardium and sclerotic changes in the aorta and walls of the small arteries (Weisskopf et al., 2009). Thus, it has been suggested that inhibition of super oxide dismutase resulting in the elevation of serum lipid peroxide, formation of atherosclerosis plaque and inhibition of the activity of cytochrome p 450 leading to an increase in serum lipids and their accumulation in vessel walls (Azab, 2014).

The effects of lead exposure with different doses on serum lipids and lipoproteins levels in either animal or human were reported to increase, decrease, or no changes (Patra and Swarup, 2000, Mezes et al., 1996, Kuzminskaya et al., 1965, Revis et al., 1980).

Metabolic and morphologic manifestations of lead exposure were compared in adult rats receiving Pb in their drinking water and calcium in diet (Mahaffey et al., 1973). Animals consuming the low calcium diet have increased blood, kidney, and femur lead levels, urinary excretion of δ-ALA, and renal intra-nuclear lead inclusion bodies at lower doses of lead than those on the adequate calcium diet (Bradberry and Vale, 2009).

Lead is known to interfere with mitochondrial energy metabolism that is necessary to reduce ferric iron to ferrous iron before insertion of iron into porphyrin ring. If there is insufficient ferrous iron for incorporation by ferrochelatase into heam, protoporphyrin accumulates (Wolff, 1994). Ferrochelatase activity is sensitive to both lead and iron. Kappor et al., 1984 have reported that the enzyme kinetics of ferrochelatase in isolated human erythrocytes change with both iron and lead concentrations. When iron deficiency is present, ferrochelatase is more sensitive to lead effects (Shah et al., 2013).

In addition to the numerous toxic effects of lead on various target organs, a number of studies have shown that acute and chronic exposure to inorganic lead may result at high levels in an immediate immune suppressive effect (Koller and Kavacic, 1974, Luster et al., 1978). But at low levels, it reported an increase (Borella and Giardino, 1990), a decrease (Koller, 1973) or no change (Routt and Charles, 1976) on immune function in experimental systems. Many factors could account for such effects, including dose of metal, chemical forms, animal strain and / or species (Michael, 2010). Thus, these controversies regarding the influence of Pb on immune system give a very vast possible domain of research (Sienra et al., 2004).

A study of the influence of vitamin C on the tissue deposition of lead in rats suggested that it might be useful as a prophylactic agent for lead poisoning (Simon, 2003). Previous studies in rats demonstrated that ascorbic acid decreases Pb intestinal absorption and increases its renal clearance (Fotherby et al., 2000). Further studies indicated that ascorbic acid may chelate lead and decrease the risk of its toxic effects on immune system; it helps this system to fight off foreign invaders and tumour cells possibly by stimulating the production of white blood cells, primarily neutrophils, which attack foreign antigens (Okediran et al., 2009). It also boosts the body’s production of antibodies, proteins that helps protect cells from viral invaders (Null, 1994). In addition to its great action against free radical produced by lead, vitamin C works along with vitamin E,
a fat-soluble antioxidant, and the enzyme glutathione peroxidase to stop chain reaction of free radicals (Ming et al., 2006).

Epidemiological studies supported by experimental data from both animals and humans, have made a significant contribution to increasing knowledge of the relationship between diet and the immune system, considering nutrient intake as a critical determinant of immune-competance (Klasing and Leshchinsky, 2000). This association was confirmed after the recognition of long chain n-3 polyunsaturated fatty acids (PUFA) as nutriments that participate in the regulation of immune system functions (Calder, 2003).

Olive Oil, a fundamental constituent of the Mediterranean diet, is also able to modulate the immune system, since it contains numerous components that possess some biological activities. Olive Oil is mainly composed of oleic acid, plus additional different chemical components such as sterols, alcohols, antioxidants and other fatty acids (palmitoleic acid, linoleic acid, alfa-linoleic acid) of minor relevance. An interesting study has demonstrated that polyphenolic substances contained in Olive Oil possess anti-inflammatory properties (Beauchamp et al., 2005).

Different studies investigating the effects of fatty acids on the immune system have demonstrated that a diet containing Olive Oil provoked proliferation in response to the mitogen concavalin A (con A) (Yaqoob et al., 1994).

In the Mediterranean region, many studies of the chemical composition of virgin Olive Oil have been carried out so far (Beauchamp et al., 2005, Ming et al., 2006) but reports on its effects on experimental animals and human biochemical parameters are few in the literature.

The aim of the present study is to assess the relationships between ascorbic acid and/or virgin Olive Oil on immune response in Pb exposed wistar rats. However, several leukocyte sub-populations, serum HDL, LDL, total cholesterol, triglycerides, calcium, iron, RBC, Hb and immunoglobulin levels have been assayed.

Materials and methods

Animals’ treatment

Five weeks old, Wistar rats, weighting an average of 100g, were obtained from Pasteur Institute, Algiers. Fourty females’ were supplied with standard diet ad-libitum. Four groups were given deionized water only (control), 500mg lead acetate (Pb), 500 mg lead acetate combined with 300 mg vitamin C (Pb-Vit C), or 500mg lead acetate combined with 5 % virgin Olive Oil (Pb-OO) for a period of four weeks. In this work, Pb and Pb-Vit C were given in drinking water, whereas Pb-OO was mixed with the diet. The experiment was carried out at the husbandry of Biology Department. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of our Institution.

Samples’ treatment

After decapitation, peripheral blood was collected in heparinised tubes, and was used for counting haematological parameters using Automatic Coulter Counter Machine (T 540). The other part of blood was collected in dry tubes and used for the measurement of serum HDL, LDL, total cholesterol (Naito, 1984), triglycerides (Buccolo et al., 1973), calcium (Bauer, 1981) and iron (Wick, 1998). The serum pools sampled for protein measurements were frozen immediately at -20°C until they were separated by cellulose acetate electrophoresis.

Statistical analysis

The one-way analysis of variance (ANOVA) was used to compare between groups. Student’s t-test was applied to compare each treated group against the control. However, the significant test was considered at p< 0.05 level.

Results and discussion

Results are shown in Fig.s 1-11. There was no difference in daily water consumption by Wistar rats.
either in Pb treated group alone, or in the combined groups when compared with the control. Leucocytes counts were significantly increased by Pb and no abnormalities were observed in Pb-Vit C and Pb-OO groups compared to the control.

The significant increase in WBC counts may indicates the clear Pb intoxication after one month exposure. Lead is known to promote oxidative damage by the formation of peroxides (Mestek et al., 1989; Mezes et al., 1996; Patra et al., 2001).

As a result free radicals over production are likely to cause dysfunction in mitochondria energy metabolism, membrane transport and possibly cell death, which could provoke an increase in inflammatory immune response against apoptosis (Patra et al., 2001).

![Fig 1. Level of WBC in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb.](image1)

![Fig 2. Level of lymphocytes in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb.](image2)

![Fig 3. Level of triglycerides in Pb exposed and control groups of Wistar rats. Significant at b: control vs Pb-Vit C.](image3)

![Fig 4. Level of cholesterol in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb.](image4)

![Fig 5. Level of HDL in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb.](image5)

![Fig 6. Level of LDL in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb, b: control vs Pb-Vit C.](image6)
Fig 7. Level of immunoglobulins in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb, b: control vs Pb-Vit C.

Fig 8. Level of calcium in Pb exposed and control groups of Wistar rats.

Fig 9. Level of iron in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb, b: control vs Pb-Vit C, c: control vs Pb-OO.

Fig 10. Level of RBC in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb.

Fig 11. Level of hemoglobin in Pb exposed and control groups of Wistar rats. Significant at a: control vs Pb, b: control vs Pb-Vit C, c: control vs Pb-OO.

During the treatment period, total cholesterol and HDL levels were significantly decreased in Pb exposed group. However, no significant variation among combined group was observed for these parameters when compared to the control. A remarkable increase in LDL-cholesterol was noticed in the present investigation, accompanied with a significant decrease of HDL-cholesterol in rats intoxicated with Pb. Earlier, Fossati and Principe, (1982) reported that the increase in the level of triglycerides of Pb treated animals may indicate the breakdown of fatty acids, where plasma LDL can undergo reuptake in the liver via specific receptors and get cleared from circulation. This increase in plasma LDL concentration may be due to a defect in LDL receptors and this explanation goes perfectly with the actual results.

On the other way, the good cholesterol “HDL” seems to help scavenge cholesterol from extra hepatic tissues. Therefore, the decreased HDL concentration can contribute to the increased cholesterol levels. Thus, an augmentation of HDL-cholesterol level was reported by Cocco et al, (2010), but this rise was accompanied by a declined total cholesterol level, which agrees with the present funding.

Whereas, the association between Pb exposure and high serum lipid levels is biologically acceptable and could be due to either increased synthesis or decreased removal of lipoproteins (Calder, 2003). The slow removal may occur because of the alteration of cell-surface receptors for lipoproteins or because of
the inhibition of hepatic lipoprotein lipase activity (Mezes et al., 1996). Furthermore, Pb has been shown to depress the activity of cytochrome P-450 in man; this can limit the biosynthesis of bile acids, which is the only significant route for cholesterol elimination from liver. Increased synthesis may be due to a lead-induced increase in hepatic enzymes at important control points for de novo cholesterol synthesis, as it was found in Wistar rats, or it may be related to impaired feedback inhibition (Shalan et al., 2005).

Results showed a reduction in the level of haemoglobulin and RBC, owing to the fact that Pb intoxication causes deficiency in haem synthesis. Thus, the obtained results agreed with those of several authors (Fischman et al., 1981; Serrill et al., 1971), because Pb pollution has an inhibitory effect on globin synthesis, inhibits iron to form haem and inhibits delta amino-levulinic acid dehydrates of red cells. Moreover, previous studies have shown that Pb toxicity facilitates the conversion of hemoglobin into methemoglobin. Though, during hemoglobin oxidation in the presence of Pb, hydrogen peroxide is generated, which may induce lipid peroxidation in the erythrocyte cell membrane (Vargas et al., 2003). As a result, Pb might induce generation of ROS by interacting with oxy-Hb, leading to peroxidative damage of erythrocyte membranes (Ribarov et al., 1981). Moreover, free radicals produced in the presence of heavy metals contribute significantly to hemoglobin denaturation and precipitation, leading to anæmia which has been seen in the actual study by a decrease in iron and haemoglobin levels after four weeks exposure.

Concerning Pb-Vit C and Pb-OO groups, they seem to attenuate Pb intoxication remarkably. Vitamin C is known as a major water-soluble antioxidant and acts as the first defence against free radicals in whole blood (Niki et al., 1988) and plasma (Frei et al., 1989). Virgin olive oil rich in α-tocopherol known to have the highest biological activity (Machlin, 1991) is the most abundant lipophilic antioxidant (Burton et al., 1983). Also, olive oil can attenuate oxidative stress reactions and diminishes apoptosis. (Flora et al., 2003; Kashif et al., 2004; Beauchamp et al., 2005).

A nutritional interaction of vitamin E and ascorbic acid in rats has been reported earlier (Chen and Barnesk, 1976), where dietary vitamin E appears to play a role in hepatic ascorbic acid biosynthesis (Carpenterm, 1959). In the Pb-treated rats, dietary vitamin E has reduced the elevation of plasma and urinary ascorbic acid (Kobayashi and Yoshida, 1986; Chowe, 1979).

In the present study, the administration of vitamin C and olive oil has reduced the changes of WBC, lymphocytes and lipid profile. The protective effects of vitamin C and vitamin E against Pb poisoning was located outside and inside the membranes, respectively, or was due to their interferences with Pb intestinal absorption. Vitamin C might increase urinary elimination of Pb, while vitamin E is an important lipid soluble antioxidant, residing in cell membranes, both scavenge reactive oxygen species generated during oxidative stress induced by Pb poisoning. (Upsani and Bataaram, 2003; Flora et al., 2003).

The immunotoxic effects of Pb have been investigated extensively (Korzeniewski, and Collewart, 1983), but few studies have included the effects of ascorbic acid supplementation on Pb immunotoxicity (Pastoret et al., 1990; Ercal et al., 2000). Pb in the present work can modulate the immune system positively, which are similar to those of Borella and Giardino (1990) who confirmed some results reported by another study (MaCabe and Lawrence, 1990), that animals exposed to Pb were susceptible to lymphocyte-stimulating characteristics, translated by a significant increase in Immunoglobulins secretion. Contrary, Pb was found to be an immunossupresseur (Koller, 1974; Koller and Kovavic, 1976). Moreover, no significant differences in serum immunoglobulin levels was found between the workers with a mean blood Pb of <2ug/dl and those with a median blood Pb of 39ug/dl (Pinkerton et al., 1998). In addition, Queiroz et al. (1993) have observed no change in immunoglobulin levels of individuals with low Pb exposure on one hand, and no correlation between serum immunoglobulins and blood Pb concentration compared to the control, on the other hand, suggesting that chronic exposure to Pb seems to
suppress the functional activity of polymorphonuclear cells. In contrast, the levels of immunoglobulins from the two combined groups (Pb-Vit C and Pb-OO) are almost similar to the control. These results are in line with those of Ercal et al., (2000) who mentioned that the administration of 5.5mmol/kg of N-acetylsystrine (NAC) in the drinking water for 1 week significantly reversed the inhibitory effects of Pb on serum immunoglobulin levels. Other comparative result which was done on some known chelators gave the evidence that the effectiveness of vitamin C is similar to those of EDTA, DMSA in protecting cells from the oxidative stress during heavy metals intoxication (Gey, 1994). Accordingly, the beneficial protective action of antioxidants in suppressing the toxic effects of Pb against immunoglobulins was documented in many studies (Calabares et al., 1987; Kashif et al., 2004; patra et al., 2001).

Calcium concentration has not been affected in all groups, but iron level was significantly decreased in all treated groups when compared to the control. Results indicate a noticeable drop in plasma iron concentration of Pb exposed rats, with no effect on calcium level. It has been reported that Pb might competes with ions such as Cu, Zn, Fe and Mg that are essential for the activity of antioxidant enzymes resulting in a decrease of Superoxide dismutase, Catalase, Acid phosphatase activities as a result of Malondialdehyde accumulation, a marker oxidative damage (Cocco et al., 2010). The level of plasma iron remained significantly low even in the presence of virgin olive oil or vitamin C, which may indicate a problem of intestinal absorption. To conclude, Pb contaminated diet containing either vitamin C or Olive Oil, could participate in reducing metal toxicity.

References


