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ERYTHROCYTIC SUPEROXIDE DISMUTASE AND CATALASE ENZYMES, LIPID PEROXIDE AND TOTAL THIOLS LEVELS IN GOATS EXPOSED TO LEAD ACETATE

(With 2 Tables and one Figure)

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نشاط خمائر سوبر أكسيد ديسميوتيز والكتاليز وكذلك البيرأكسيد الدهني والثيولات الكلية لكرات الدم الحمراء فى الماعز المتعرض لخلات للرصاص

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في هذه الدراسة تم إستخدام خمس عشرة حيوانا من الماعز (إناث بالغة من العمر ثلاث سنوات) قسمت إلي مجموعتين، الأولي منها أحتوت علي خمسة حيوانات وتم أستخدامها كضابط للتجربة والثانية منها أحتوت علي عشر حيوانات تم تعرضها للرصاص في صورة خلات رصاص عن طريق الفم بجرعة ٩و٩ مجم / كجم من وزن الحيوان لمدة سبع أسابيع. تم جمع عينات الدم في بداية التجربة للمجموعة الأولي و في المجموعة الثانية تم جمعه أسبوعيا علي النحو التالي يوم ٧،١٤،٢١،٢٨،٣٥،٤٢،٤٩. وقد تم قياس كل من السوبر أكسيد ديسميوتيز والكتاليز والبيرأكسيد الدهني والثيولات الكلية في محلول ١٠% لكرات الدم الحمراء. كما تم قياس مستويات الرصاص والنحاس والزنك والحديد في الدم. وقد أظهرت النتائج ما يلي:ـ (١) إختزال في نشاط السوبر أكسيد ديسميوتيز في اليوم السابع وظل منخفضا حتي اليوم الثامن والعشرين ثم تبعه زيادة في النشاط من اليوم الخامس والثلاثين حتي نهاية التعرض (اليوم ٤٩). (٢) زيادة في النشاط أنزيم الكتاليز في اليوم السابع ثم تبعه إنخفاض حتي اليوم التاسع والأربعين. (٣) زيادة في مستوي البيرأكسيد الدهني في اليوم الرابع عشر وظلت الزيادة حتي اليوم التاسع والأربعين. (٤) إنخفاض الثيولات الكلية إبتداءا من اليوم السابع وأستمراره حتي نهاية التجربة (اليوم التاسع والأربعين). (٥) إرتفاع تركيز كل من الرصاص والحديد صاحبه إنخفاض تركيز النحاس والزنك في الدم. من هذه الدراسة تبين أن التعرض للرصاص عن طريق الفم والذي أدي إلي إختزال مستويات الإنزيمات الدفاعية المضادة للأكسدة لكرات الدم الحمراء (السوبر أكسيد ديسميوتيز والكتاليز) والثيولات الكلية والزيادة في البيرأكسيد الدهني يظهر دور المشتقات الحره في عملية التسمم بالرصاص.

SUMMARY

Fifteen adult female goats were used in this study to evaluate the effect of lead on erythrocytic antioxidant enzymes, lipid peroxide and total thiols levels as well as elemental status of the whole blood. These goats were classified into two groups, first (5 goats were not exposed to lead and served as control), and second (10 goats were exposed orally to lead as lead acetate in a dose of 9.9 mg Pb per kg body weight for 49 days). Erythrocytic haemolysate (10 percent) was prepared and analyzed for superoxide dismutase (SOD), catalase, lipid peroxide (LPO) and total thiols (T-SH). Lead, copper, zinc and iron were also estimated in the whole blood. The results of this study revealed that: **(1)** Reduction in the activity of SOD by day 7 and remained lower until day 28 followed by an increase on day 35 upto day 49. **(2)** An increase in the catalase activity on day 7 followed by decrease until day 49. **(3)** Lipid peroxide level was recorded to be higher by day 14 and remained until day 49. **(4)** Total thiols showed a decrease from day 7 of exposure until the end of experiment. **(5)** An increase in lead concentration accompanied by an increase in iron level while copper and zinc showed a decrease in their concentrations. From this study we can conclude that oral exposure of goats to lead reduced the erythrocytic antioxidant defense enzymes (SOD and catalase), total thiols content and increased lipid peroxide level may play a part in the increased membrane lipid peroxidation and explain the possible role of free radicals in the pathogenesis of lead toxicity.

Key words: Lead- SOD- catalase- total thiols- copper- zinc.

INTRODUCTION

Heavy metals are among the most widespread potential chemical contaminants in the environment and are transferable to man and animals through diet and other routes (Pace and Lannucci, 1994). Toxic effects of lead are manifested by lead encephalopathy, gastroenteritis and degeneration of peripheral nervous system and range from overt clinical signs to subclinical subtle effects (Radostitis *et al.*, 1995 and Swarup, 1996).

Various mechanisms explain the lead-induced toxicity such as interaction of lead with bioactive ligands resulting in inactivation of several vital enzyme systems, disturbances in mineral metabolism and

demyelination of nervous tissue (Valle and Ulmer, 1972; Klassen, 1985; Ercal *et al.*, 2001). Oxidative damage has been proposed as another possible mechanism involved in lead toxicity (Adonaylo and Oteiza, 1999; Patra and Swarup, 2000). Some studies both in vitro and in vivo, and for occupationally exposed workers suggest that at least some lead-induced damage may occur as a consequence of the propensity of lead to disturb the delicate prooxidant and antioxidant balance that exists in mammalian cells (Lima-Hermes *et al.*, 1991; Monteiro *et al.*, 1985; Donaldson and Knowles, 1993; Ercal *et al.*, 2001).

In workers who occupationally exposured to lead, the stimulation of lipid peroxidation and decrease of blood SOD activity as well as increase of activity of this enzyme was found (Ito *et al.*, 1985). Lipid peroxides, as well as triglycerides have become lately the subject of numerous investigations concerning arteriosclerosis and risk factor of cardiovascular diseases (Watts, 1990). Lipid peroxidation occurs when free radicals are generated adjacent to polyunsaturated fatty acids (PUFA) as arachidonic and linolenic acids in membrane lipids. The reactive radical will abstract a hydrogen atom from one of the =CH groups in the fatty acid to generate a carbon-centered radical within the membrane. Carbon-centered radicals will combine with molecular oxygen and produce peroxyl radicals. Therefore, the net results of one very reactive radical species attack upon the membrane is to convert PUFA into lipid hydroperoxides. These lipid hydroperoxides tend to migrate a way from the hydrophobic interior of the membrane to the surface, thus disrupting membrane organization. Peroxidation of biological membranes increase their leakiness to ions and causes damage to trans-membrane proteins such as receptors and enzymes. LPO decompose in the presence of iron and copper ions to form a wide range of cytotoxic aldehydes, such as malondialdehyde (MDA) and hydroxynonenal, which themselves are capable of chemically modifying proteins and DNA (Ward and Peters, 1995).

The process of LPO formation plays an important role in the pathogenesis of several status including aging, cancer, atheroscalerosis, viral infection arthritis and cataracts. Initiation of LPO is solely carried out by free radicals such as superoxide, hydroxyl radical and H_2O_2 causing cellular injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharides as well as protein cross-linking and fragmentation. This disturbance results in membrane structure changes such as fluidity, transport and antigenic character (Slater, 1984).

Lead ions accelerate the lipid peroxidation observed when Fe^{2+} ions are added to phospholipid liposomes at pH 5.5 or 7.4, although Pb^{2+} ions alone do not induce any peroxidation. Pb ions accelerate the peroxidation of erythrocytes induced by a high concentration of H_2O_2 in the presence of azide and they also increase the peroxidation that occurs when Fe^{2+} or Fe^{2+}-adenosine diphosphate is added to rat liver microsomes at pH 7.4. It has been proposed that increased lipid peroxidation may contribute to the toxic action of Pb^{2+} in humans (Quinlan *et al.*, 1988).

The aim of the present work was to elucidate the subacute toxic effect of oral exposure to lead acetate on erythrocytic superoxide dismutase and catalase enzymes, lipid peroxide and total thiols levels as well as elemental status of the whole blood in goats.

MATERIALS and METHODS

Collection of samples:

Blood samples were collected at start of the experiment in the first group, while in the second group it were collected after weekly intervals (i.e. on day 7, 14, 21, 28, 35, 42 and 49).

Preparation of RBCs haemolysate:

Freshly collected blood samples were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. The sediment cells were washed with saline solution. This process was repeated three times. Washed erythrocytes were haemolysed with 9-fold volume of distilled water to prepare 10% RBCs haemolysate.

Estimation of enzyme activity:

Esimation of SOD activity based on its ability to inhibit the autooxidation of epinephrine in an alkaline medium (pH 10.2) according to Misra and Fridovich (1972). Catalase activity was estimated according to the method described by Cohen *et al.* (1970) using spectrophotometer (Milton Roy spectronic 1201, USA) at wave length 240 nm. The reaction was started by addition of 50 µl of dilute sample to 3 ml of phosphate buffer H_2O_2 solution. Initial absorbance was read after 20 second against distilled water instead of H_2O_2. The time required for initial absorbance to decrease by 0.05 unit was noticed. Catalase activity was calculated and expressed in units/mg Hb.

Estimation of lipid peroxides and total thiols:

Malondialdehyde (MDA) concentrations are considered to be an index of the peroxidation of the lipid membrane. Lipid peroxides were

colormetrically measured using commercial kits according to the method of Esterbauer and Cheeseman (1990). Protein concentrations were measured according to the method of Bradford (1976). Total thiols were measured according to the method of Ellman (1959).

Metals estimation in the whole blood:

Standard procedures were used to estimate lead, copper, zinc and iron in the blood. All glassware, pipette tips and plastic ware were rinsed with 25% HNO_3 to avoid metal contamination. 5ml blood were used and digested using concentrated nitric acid and perchloric acid (2:1). Lead, copper, zinc and iron were measured according to Yeager *et al.* (1971), Parker *et al.* (1968) and Agemain *et al.* (1980) respectively using atomic absorption spectrophotometer (Buck Model 210 VGP, USA)

Statistical analysis:

Data was analysed statistically using student *t*-test. Probability values 0.05 and 0.001 were considered statistically significant (Snedecor and Cochran, 1967).

RESULTS

The results of this study are summarized in tables 1 and 2.

DISCUSSION

Lead is one of the most abundant heavy metals in the environment. Inorganic lead compounds enter the organism via inhalation or ingestion. Only organic lead compounds penetrate intact skin. At moderate levels of exposure, lead may induce biochemical and functional changes as it interferes with proper function of cellular membranes and enzymes, owing to the formation of complexes between lead and ligands containing S, P, N, O as -SH, $-H_3$, PO_3, NH_2, -OH groups (Tsalev and Zaprianov, 1985).

Some animal studies have indicated that lipid peroxidation is enhanced in target tissues of rodents exposed to lead compounds. Levander *et al.* (1977), showed erythrocyte TBA (thiobarbituric acid)-chromogen production in brain homogenates of adult rats exposed to lead compounds for 10 days.

Epidemiological studies on workers with occupational exposure to lead and experimental studies on rats injected with lead indicate that lead exposure increases serum lipid peroxide and inhibits blood SOD activity (Ito *et al.*, 1985; Skoczynska and Smolik, 1994). Using an in

vitro assay system, the addition of lead at higher than 20 µM concentrations to untreated rat liver microsomes was found to increase NADPH-dependent lipid peroxidation, (Xiao *et al.*, 1989), and this lead concentration inhibited bovine SOD activity. On the bases of these results it is proposed that the increase in serum lipid peroxide levels following exposure to lead is not only due to the stimulation of lipid peroxidation but also to the inhibition of SOD activity (Ito *et al.*, 1985). The reduction in SOD activity may be due to direct lead ions activity and decrease in the copper concentrations, given the well-known antagonistic relationship between lead and copper (Skoczynska *et al.*, 1993).

Interference in haem synthesis through inhibition of delta aminolevulinic acid dehydratase lead to increase of D-ALA which considered as one of the important biochemical effects of lead (Moore, 1986). The coupled autooxidation of oxyhemoglobin and D-ALA generate active oxygen species resulting in oxidative stress (Monteiro *et al.*, 1986). SOD, catalase and glutathione peroxidase are the major enzymes present in RBCs to counteract the toxic effects of reactive oxygen species such as superoxide radicals and hydrogen peroxides (Moral *et al.*, 1977). Lead enhances generation of superoxide radicals (Medeiros *et al.*, 1983) and has no direct inhibitory effect on activity of bovine SOD (Mylroie *et al.*, 1986). Meanwhile Patra and Swarup (2000) found diminished activity of SOD after oral exposure to lead in calves. In the present study, the reduction of SOD activity might have occurred due to over utilization of SOD in neutralizing excess superoxide radicals. Results in the present study are in agreement with those reported by Patra and Swarup (2000), and contradict Ito *et al.* (1984) and Monteiro *et al.* (1986) as they found high level of erythrocytic SOD and glutathione peroxidase in lead exposed workers. Several air pollutants have been reported to enhance the oxidation of oxyhemoglobin to methemoglobin and thus generate superoxide ions, which in turn diminish the activities of SOD followed by induction of its biosynthesis as a protective mechanism against free radical toxicity (Fridovich, 1984).

Catalase is responsible for breakdown of hydrogen peroxide produced during metabolism. Reduced activity of catalase in the present study could be attributed to increased generation of hydrogen peroxide in lead exposed goats due to accumulation of D-ALA (Medeiros *et al.*, 1983). Ariza *et al.* (1998) found that lead has no direct effect on catalase activity, but some reports recorded effects of lead on catalase activity

both in vivo and in vitro (Valenzuela *et al.*, 1989; Somasekharaiah *et al.*, 1992 and Patra and Swarup, 2000).

Richness of PUFA continual exposure to high concentration of oxygen as well as the presence of powerful transition metal catalyst make the erythrocytes highly susceptible to oxidative damage (Clemens and Waller, 1987). Increase of LPO in the this study reflects the higher production of peroxyl radicals resulting in peroxidation of PUFA. Results recorded in this study are in agreement with that obtained from the study of occupationally workers (Ito *et al.*, 1985; Sugawara *et al.*, 1991 and Jiun and Hsien, 1994), chick embryo (Somasekharaiah *et al.*, 1992), calves (Patra and Swarup, 2000) and rats (Aykin-Burns *et al.*, 2003) after exposure to lead.

Decrease in sulfhydryl groups in the present study is in agreement with that reported by Patra and Swarup (2000) in lead exposed calves. However, decreased glutathione concentration was found in blood of pregnant women exposed to lead and cadmium (Tabacova *et al.*, 1994). Reduced sulfhydryl level in RBCs hemolysate in goats exposed to lead in the present study may be attributed to increased activity of glutathione peroxidase in the oxidative stress conditions. Thiol containing biomolecules play vital role in chelating lead and counteracting its toxic effects (Leeming and Donaldson, 1984; Munoz *et al.*, 1993).

In the present study, a depletion of copper and zinc with elevation of iron as a result of exposure to lead acetate was recorded. These obtained results are in agreement with the results recorded by Skoczynska *et al.* (1993) and Skoczynska and Smolik (1994) who found an increase in copper and zinc concentration in rats exposed to lead.

From this study it could be concluded that oral exposure of goats to lead acetate reduced the erythrocytic antioxidant defense enzymes (SOD and catalase), total thiols content and increased lipid peroxide level which may play a part in the increased membrane lipid peroxidation and indicating the possible role of free radicals in the pathogenesis of lead toxicity.

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Table 1. SOD, catalase, LPO and total thiols levels of erythrocyte haemolysate of goats exposed to lead (mean ± S.E.)

Exposure time (days)	SOD (U/mg protein)	Catalase (U/mg Hb)	LPO (nmol MDA/mg protein)	Total thiols (nmol/mg protein)
7	2.57 ± 0.11*	4.95 ± 0.21**	1.63 ± 0.03**	30.78 ± 1.39*
14	2.56 ± 0.13*	2.86 ± 0.13**	1.83 ± 0.03**	29.27 ± 0.96**
21	2.62 ± 0.11*	2.93 ± 0.14**	2.00 ± 0.08**	27.95 ± 0.91**
28	2.54 ± 0.11*	2.97 ± 0.12**	2.06 ± 0.03**	27.64 ± 0.88**
35	2.76 ± 0.14	3.06 ± 0.15**	2.03 ± 0.04**	25.93 ± 0.79**
42	2.90 ± 0.14	3.10 ± 0.11**	2.06 ± 0.04**	25.81 ± 0.78**
49	3.00 ± 0.13	3.07 ± 0.06**	2.06 ± 0.03**	25.67 ± 0.77**
Control	3.01 ± 0.11	3.88 ± 0.14	1.43 ± 0.03	36.46 ± 1.16

*: Significant difference from control at $p \leq 0.05$.

**: Significant difference from control at $p \leq 0.001$.

Table 2. Blood lead, zinc, copper and iron concentrations (ppm) in goats exposed to lead (mean ± S.E.).

Exposure time (days)	Lead	Zinc	Copper	Iron
7	0.58 ± 0.04**	5.30 ± 0.39	0.85 ± 0.04*	4.62 ± 0.28*
14	0.76 ± 0.04**	4.48 ± 0.29*	0.74 ± 0.04**	4.77 ± 0.26*
21	0.86 ± 0.05**	4.31 ± 0.27*	0.64 ± 0.04**	4.84 ± 0.19**
28	0.97 ± 0.04**	4.27 ± 0.24*	0.64 ± 0.04**	5.04 ± 0.26**
35	1.16 ± 0.05**	4.21 ± 0.25*	0.65 ± 0.03**	5.14 ± 0.21**
42	1.20 ± 0.04**	4.29 ± 0.25*	0.64 ± 0.03**	5.15 ± 0.19**
49	1.33 ± 0.04**	4.29 ± 0.25*	0.65 ± 0.03**	5.23 ± 0.28**
Control	0.09 ± 0.01	6.41 ± 0.55	1.01 ± 0.04	3.67 ± 0.13

*: Significant difference from control at $p \leq 0.05$.

**: Significant difference from control at $p \leq 0.001$.

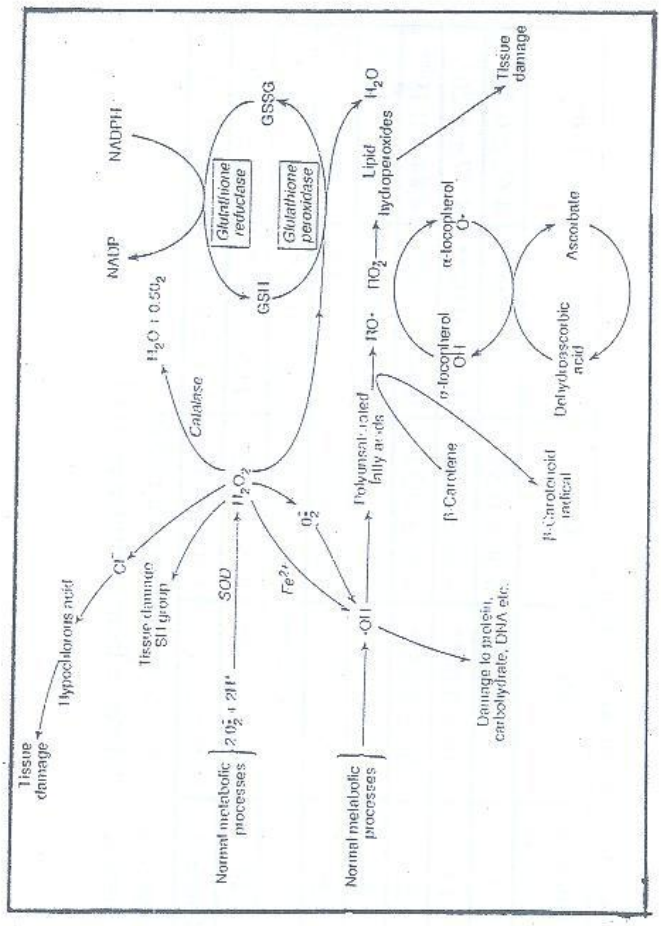

Fig. 1. Interrelationships between cytoprotective enzymes, antioxidants and free radicals. SOD (superoxide dismutase), $RO^{\cdot}$ (alkoxyl), O_2^{-} (superoxide ion), GSH (reduced glutathione, and GSSG (oxidized glutathione) (Ward and Peters, 1995).