

ANTIOXIDANT STATUS AND HEPATIC LIPID PEROXIDATION IN ALBINO RATS EXPOSED TO CEMENT DUST

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Abstract:

The present study reports the enzymatic and non-enzymatic antioxidants status as well as lipid peroxidation in albino rats exposed to cement dust for 14days and 28days respectively. The antioxidant enzymes: superoxide dismutase and catalase activity were decreased by 46% and 61% in the group exposed to cement dust for 14days (group 2) while it decreased by 55% and 64% in group 3 exposed to cement dust for 28days. The level of serum Vitamin A, C and β -carotene were significantly decreased ($p < 0.001$) in the groups exposed to cement dust for 14days and 28days respectively. Glutathione level and Glutathione s-transferase activity were decreased by 53% and 48% in group 2 while it decreased by 58% and 63% in group 3. Cement dust also elicited lipid peroxidation in both groups by 61% and 63% respectively. This investigation reveals an alteration of enzymatic and non enzymatic antioxidant status and induction of lipid peroxidation by cement dust. This study has provided further confirmation that component of particulate matter causes oxidative damage.

Keywords: Cement, antioxidants status, lipid peroxidation, enzymatic antioxidants, non-enzymatic antioxidants

1.0 Introduction

The continuous chaotic exit of industrial wastes into the atmosphere has been established by researchers to be one of the great dangers being done to the environment and the totality of beings. One of these pollutants is cement dust of which the most popular is Portland cement. The cement industry is considered as a major pollution problem because of dust and particulate matter emitted at various steps of cement production. Cement dust consists of many toxic heavy metals like nickel, cobalt, lead, chromium and Silica which are pollutants hazardous to the biotic environment, with adverse impact for vegetation, human and animal health and ecosystems (Baby et al.2008).

Occupational cement dust exposure has been associated with an increased risk of liver abnormalities, pulmonary disorders, and carcinogenesis. Decreased antioxidant capacity and increased plasma lipid peroxidation have been posed as possible causal mechanisms of these diseases (Aydin et al. 2010). Exposure to cement dust facilitates the reactions in which free radicals are produced in different parts of the body. The components of cement such as silica and chromium pose danger to cellular components such as proteins, nucleic acid, DNA and membrane lipids. Silica is one of the most documented workplace contaminants. Long-term exposure to silica has been reported to be the principal cause of silicosis. A recent review (Driscoll et al., 2005) estimates 8,800 silicosis deaths a year worldwide. Occupational exposure to dust containing crystalline silica occurs in mining, ceramics production, cement production etc. (ATS,1997). Because the body cannot clear or metabolize a desirable portion of inhaled mineral dust particles, fibrosis develops in the upper regions of the lungs, which interferes with their normal expansion. Alveolar macrophages are destroyed, with fibrotic nodules forming around them. Alveolar and interstitial macrophages are activated after particle uptake and produce reactive oxygen species (ROS).

The population most exposed to cement dust pollution includes workers and managers in cement plants and factories, families of workers and managers living in staff houses of factories, and other neighbourhood habitations. Children studying in the schools situated in proximity to factories are particularly prone to cement dust exposure. Several studies have demonstrated linkages between cement dust exposure, chronic impairment of lung function and respiratory symptoms in human population. Cement dust irritates the skin, the mucous membrane of the eyes and the respiratory system. Its deposition in the respiratory tract causes a basic reaction leading to increased pH values that irritates the exposed mucous membranes (Zelege et al. 2010). A study to evaluate the mutagenic effect of occupational

exposure to cement dust in some workers concludes that the chromosomal damage was more pronounced in the workers who are also smokers when compared with the non – smokers both in the control and exposed group (Fatima et al. 2001).

The aim of this study therefore is to investigate the effect of cement and its particulate matters on lipid peroxidation and antioxidant status in albino rats exposed to cement dust for 14 and 28 days respectively.

2.0 Materials and methods

2.1 Chemicals

1,2dichloro-4-nitrobenzene (CDNB) was purchased from Sigma-Aldrich with product code 100913730 and identification code no 237324-10G. Thiobarbituric acid, 5,5' Dithiobis-2-nitrobenzoic acid (DTNB), Ethylene di tetraacetic acid (EDTA), Glutathione and hydrogen peroxide were all purchased from Sigma Chem Co. (USA). All other chemicals were of analytical grade.

2.2 Animals and Treatment

Male Albino rats (Wistar Strain) weighing between 160g-200g were used for the study. The animals were obtained from the animal house of the Veterinary Research Institute Vom, Jos. The animals were fed with standard food pellets and liberally supplied with water. Thirty rats were divided into three groups of 10 animals each. The first group (group 1) was used as control (They were not exposed to cement dust) while the second group (group 2) and third group (group3) were exposed to cement dust for 14days and 28days respectively.

2.3 Preparation of Serum and Liver Homogenate

The rats were sacrificed by cervical dislocation and blood was collected from the rats by cardiac puncture. Serum was prepared by centrifugation for 10minutes at 3000xg in an MSC bench centrifuge. The clear supernatant was used for the estimation of serum enzyme. The liver was removed and rinsed in ice-cold 1.15% KCl and weighed. It was then homogenised in a Tris-sucrose buffer (10mM Tris, 1.25M Sucrose, 1mM EDTA, pH 7.4). The homogenate was centrifuged at 10,000xg for 20minutes to obtain the supernatant fraction which is used in the assessment of Lipid peroxidation.

2.4 Determination of antioxidant enzyme activities

Activity of Catalase was determined according to the method of Singha (1972). Superoxide dismutase activity was determined by the procedure of Misra and Fridovich by measuring the inhibition of autooxidation of epinephrine at pH 10.2 at 30⁰C.

2.5 Assay of non enzymatic antioxidants

Serum Vitamin C was determined chemically according to the procedure described by Erel et al. (1997) using dinitrophenylhydrazine (DNPH). Serum Vitamin A and B-Carotene were assayed according to the method of Suzuki and Katoh (1990) as described by Kokcam and Naziroglu (1999).

2.6 Assessment of Lipid Peroxidation

Lipid peroxidation was assessed in the liver homogenate by measuring the thiobarbituric acid reactive substances (TBARS) at 532nm according to the method of Varshney and Kale (1990).

2.7 Determination of glutathione and Glutathione s-transferase

Glutathione was determined in the supernatant fraction of liver homogenate according to Jollow et al. (1974). Glutathione s-transferase activity was determined by the method described by Gibson and Skett (1994) using 1,2-dichloro-4-nitrobenzene as substrate.

2.8 Protein Determination

Protein content of all fractions was estimated by the method of Lowry et.al. (1951) using bovine serum albumin as standard.

2.9 Statistics

The data were analysed by a two tailed student's t-test. P values less than 0.001 were considered statistically significant between control and cement exposed animals.

3.0 Results

Table 1 shows the results of exposure to cement dust for fourteen and twenty eight days on cytosolic superoxide dismutase and catalase activities. Exposure to cement dust for 14 days (group 1) was found to decrease both superoxide dismutase and catalase activities by 46% and 61% respectively. There was a further reduction in the activities of these enzymes for the group 2 exposed to cement dust for twenty eight days (28) by 55% and 64% respectively. Although there was a further reduction in the activities of these enzymes in group 3, the reduction was not significant ($p > 0.001$) when compared with group 2.

Table 1 also represents the effect of exposure to cement dust on non enzymic antioxidants. Serum Vitamin A, Vitamin C and β - Carotene were found to decrease for the groups exposed to cement dust for 14days and 28days respectively. Vitamin A was found to decrease by 37% in group 2 while there was a 50% decrease in the level of Vitamin A in group 3. Vitamin C and β -carotene both decreased by 46% and 18% for group 2 and 43% and 30% for group 3.

Table 1. Effect of Cement dust on antioxidant enzymes and serum Vitamin A, C and β -Carotene in rats

Parameters	Control (Group 1)	Exposed to cement dust for 14 days (Group2)	Exposed to cement dust for 28 days (Group 3)
Superoxide dismutase($\mu\text{mol}/\text{mg}$ protein)	4.04 $\pm$ 0.8	2.15 $\pm$ 0.5*	1.81 $\pm$ 0.4*
Catalase (($\mu\text{mol}/\text{mg}$ protein)	2.28 $\pm$ 0.7	0.87 $\pm$ 0.3	0.8 $\pm$ 0.1*
Vitamin A ($\mu\text{mol}/\text{Litre}$)	2.22 $\pm$ 0.5	1.4 $\pm$ 0.3*	1.1 $\pm$ 0.3*
Vitamin C ($\mu\text{mol}/\text{Litre}$)	44.6 $\pm$ 3.5	23.71 $\pm$ 3.6*	25 $\pm$ 2.4*
B-Carotene($\mu\text{g}/100\text{ml}$)	10.85 $\pm$ 2.1	8.9 $\pm$ 0.7*	7.5 $\pm$ 0.5*

The values are the mean $\pm$ SD for 10 rats in each group

*Significantly different from control $p < 0.001$

Table 2. Effect of Exposure to cement dust on Glutathione, glutathione s-transferase activity and Lipid Peroxidation in rats.

Parameters	Control (Group 1)	Exposed to cement dust for 14 days (Group2)	Exposed to cement dust for 28 days (Group 3)
Glutathione (nm/mg protein)	4.3±0.5	2.0±0.2*	1.8±0.3*
Glutathione s-transferase (nmol/min/mg protein)	3.32±0.6	1.7±0.5	1.2±0.1*
Lipid Peroxidation	163.8±18.7	449.1±47.3*	483±28*

The values are the mean ± SD for 10 rats in each group

*Significantly different from control $p < 0.001$.

The effect of exposure to cement dust on Glutathione, Glutathione s-transferase and Lipid peroxidation are presented in Table 2. Glutathione and Glutathione transferase were decreased by 53% and 48% respectively in group 2. At the end of twenty eight days (group 3) Glutathione activity decreased by 58% while Glutathione s-transferase activity was reduced by 63%. Malondialdehyde level was increased by 61% in the group exposed to cement dust for 14 days while it increased by 65% in the group exposed to cement dust for 28 days.

4.0 Discussion

For many centuries polluted air has been considered to be hazardous to health and concern has been mounting during the last few decades about the possible hazardous effect of waste substances introduced into the environment daily. Many of these compounds can chemically alter DNA which in turn can lead to deleterious consequences (Calistrusjudge et al., 2002).

Our data shows that exposure to cement dust affects both the enzymatic and non enzymatic antioxidants as well as causes membrane lipid peroxidation. The result indicates a decrease in superoxide dismutase level. This could be due to the fact that exposure to cement dust generate an increase in the production of reactive oxygen species especially superoxide anion. The superoxide radical produced is neutralised by superoxide dismutase and the decrease in the level of this antioxidant reveals that exposure to cement dust leads to increased oxidative stress (Ayidin et al.2004).

The result further indicated that exposure to cement dust for 14days and 28 days decreased catalase activity. It is generally believed that hydrogen peroxide can be detoxified by catalase which removes it when present at high concentration and glutathione peroxidase which destroys it when present at a steady state (Casado et al. 1995). The reduction in the level of this enzyme may render the liver more susceptible to hydrogen peroxide induced oxidative stress.

Vitamin A, C and β -Carotene are important antioxidant vitamins that can directly scavenge free radicals. The result shows that Vitamin A, C and β -Carotene was reduced in the group exposed to cement dust for 14days and 28days respectively. The carotenoids also known as vitamin A functions as a radical trapping antioxidant. The efficient biological radical trapping antioxidant activity of carotenoid was demonstrated through its inhibition of lipid peroxidation induced by xanthine oxidase system (Krinsky and Denase 1982). Vitamin A usually protects cells and tissues against damage by reacting with radicals and being consumed in the process thereby acting as sacrificial radical trapping antioxidants (Farombi and Britton 1999a, b). Vitamin C also known as ascorbic acid on the other hand is the first line of antioxidant defense (Frei et al. 1988, 1989) and this vitamin is susceptible to free radical oxidation. Ascorbic acid functions as an important component of cellular defence against oxygen toxicity and lipid peroxidation caused by free radical mechanism (Procter and Reynolds, 1984). β -Carotene, a precursor of Vitamin A has been found to be an effective quenchers of reactive oxygen species (Burton and Ingold 1984). The apparent decrease in this non-enzymatic antioxidant observed in this study for the two groups exposed to cement dust

for 14days and 28days respectively reflects the fact that the groups exposed to cement dust suffers from oxidative injury. The level of free radical generated by exposure to cement dust has overwhelmed the antioxidant capability of the cell to protect against free radical mediated damage.

The level of reduced glutathione is a measure of the cellular redox status (Chance et al. 1979). Hence alteration in glutathione level may affect the overall redox status of the cell. The result shows a decrease in glutathione and glutathione s-transferase for the two groups exposed to cement dust. Aniya and Naito (1993) had reported that severe oxidative stress might result in decrease in glutathione s-transferase as well as glutathione. This further confirms that there is an appreciable level of oxidative stress in the groups exposed to cement dust. There was also an increase in lipid peroxidation measured as malondialdehyde released. Catalase is usually inactivated by hydrogen peroxide and superoxide radicals. Reduction in residual catalase level confers increased susceptibility on the cell to undergo lipid peroxidation. This increase in lipid peroxidation is in agreement with the study carried out on workers exposed to cement dust. (Orman et.al. 2005). Although exposure of animals to cement dust for 28days further decreased the activity and level of both the enzymatic and non-enzymatic antioxidant as well as increased further the peroxidation, these changes were not significant when compared to the groups exposed to cement dust for 14 days ($p>0.001$). The present observation suggests that the profile of enzymatic and non-enzymatic antioxidant is altered by exposure to cement dust. There is a significant association between particulate air pollution and biomarkers of oxidative stress. These associations suggest that personal exposure to fine particles in ambient air can lead to changes and damage to several components of the cell. This therefore calls for stringent regulation of environmental exposure to cement dust as well as conducting periodical studies to provide useful data such as internal exposure doses and its attendant biological effect.

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