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Research Article

EFFECT OF SILICA DUST ON ANTIOXIDANT ENZYMES IN GRANITE WORKERS

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ABSTRACT

Elemental form of silica reactive with various elements and forms silicates; however, combines either with oxygen to form silica (SiO_2). Silica and silicates represent the majority of most kinds of rocks, clays and sands. Exposure to huge quantities of free silica is odourless, non-irritant, and does not produce any instant remarkable consequences. Globally adverse effects silica has raised and alarming public health concern and reported that over two million in Europe, twenty-three million silica-exposed people in China, three million in India, and in Thailand. The global burden of quarry industries is substantial; especially, occupational hazards and illnesses in developing countries. In most of the countries, the construction industry contributes significantly to gross domestic product. It plays a vital role in terms of socioeconomical development of the country. In the 19th century, crushed stone dust was reported as potential material of choice for building durable roads, and constructions. Study results evidenced that most of the workers were not using protection equipment for better safety. It is far away from the occupational health and safety measures. Most of the equipment's were made with fabric and low-quality material. Smoking habit of workers (37.9) and controls (66.7%) and was nearly 24-26 cigarettes per day. The catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase activity levels were significantly depleted ($p<0.0001$) in exposed workers when compared with their respective control group. Data indicated that induction of oxidative stress and activation of immune system was associated with crystalline silica exposure.

Keywords: Silica, Granite Workers, Catalase, Glutathione Peroxidase, Glutathione Reductase and Superoxide Dismutase.

INTRODUCTION

The distribution of silica in nature is much like that of carbon in organic matter. The earth's crust incorporates about 12% free crystalline silica and silicon is the second one most common element after oxygen, consequently exposures to crystalline silica dust would possibly arise in a huge form of occupations, which include quarry, metallic foundries, constructions and ceramic, pottery industries. Crystalline silica is one of the most hazardous occupational risks. Crystalline silica exposure lead to silicosis further chronic exposure causes cancers. Over two million US workers have been exposed to silica dust in lots of places of work in 2003. Elemental form of silica reactive with various elements and forms silicates; however, combines either with oxygen to form silica (SiO2). Silica and silicates represent the majority of most kinds of rocks, clays, and sands. Exposure to huge quantities of free silica is odourless, non-irritant, and does not produce any instant remarkable consequences. Globally adverse effects silica has raised and alarming public health concern and reported that over two million in Europe, twenty-three million silica-exposed people in China, three million in India[1-6].

The global burden of quarry industries is substantial; especially, occupational hazards and illnesses in developing countries. In most of the countries, the construction industry contributes significantly to gross domestic product. It plays a vital role in terms of socio economic development of the country. In the 19th century, crushed stone dust was reported as potential material of choice for building durable roads, and constructions. In due course of time, trap rock, metamorphic rock and limestone, granite, sandstone and shale were used for laying roads and other construction sectors[7]. In Turkey, eighty-two silicosis and silico-tuberculosis instances out of a hundred and fifteen occupational lung diseases had been recorded in 2014 consistent with the latest Statistical Yearbook of Ministry of Labour and security of Turkey. These statistics produced from most effective workers included by using the social security system. Further to that, ceramic industry is a region with excessive contribution to employment in Turkey. The industry employs approximately 220,000 people[8]. Workers are occupationally exposed to free crystalline silica dust at some stage in many operations and crystalline silica dust ranges vary in unique ceramics processing. Elevated levels of silica were reported at different operations such as heating, moulding, and blending. Workers exposed persistently to silica dust as a result, there is a want for dependable biomarkers to predict the probability of silicosis and hazard development. Respirable crystalline silica (quartz and cristobalite) inhaled from occupational sources has been reclassified as a human carcinogen in 1997 by the international agency for research on cancer[9] (IARC, 1997). Workers of granite, ceramic cement industries are potentially exposed to silica, lime, inorganic lead, beryllium and aluminium.

Biological responses and phytochemical properties of crystalline silica might be stimulated by inherent nature of silica dust which leads to generation of reactive oxygen species and oxidative stress. In general, free radicals are generated during metabolism, exogenous exposures and their mediated effects vary from affecting cellular signalling and responses to impaired proteins, lipids, and DNA[10]; antioxidants as the frontline immune defense against free radical injury. Superoxide dismutase (SOD) causes the dismutation of superoxide radicals to form hydrogen

peroxide, subsequently, decomposed in to water and oxygen by catalase (CAT). This process prevents the hydroxyl radical's formation. Impairment of antioxidant system would lead to lipid peroxidation[11]. Silica is one of the environmental substances that cause autoimmune dysfunction.

According to the recent reports, crystallin silica is the most common sources of exposure and causes many health hazards[12,13]. However, hazards of occupational silica-containing dust exposure have been investigated and reported in various industries such as granite and stone crushers, coal miners, foundry and pottery, cement, and sand. In developing countries, occupational exposure to silica dust is still considered as a significant health problem among granite workers[14]. Silica adverse effects in granite quarry workers is not well established at study area. Hence, the study intended to examine the alterations of chronic exposure to silica on antioxidant defense enzymes in occupational workers. The study was aimed to investigate the devastating effects of occupational silica on vital oxidative stress parameters such as activities of CAT, SOD, glutathione peroxidise (GPx), glutathione reductase (GR), in erythrocytes.

MATERIAL AND METHODS

The exposed group consists of male workers (n = 29) from a granite quarry industry exposed to crystalline silica-containing dust. Control group consists of male office workers (n = 24) without dust exposure. Control group comparable for smoking habits and age to the workers were included. Male subjects of the same region were selected in order to mitigate the effects of genetic and other factors related to lifestyle habits. All the subjects were explained about the research and consent was obtained from each subject. All the procedures are approved by the St Theresa international college, institutional ethics committee (STIC/TH/PH/04/2019).

Primary data was collected by the author with the help of Thai-native speaker through personal interactions with the subjects. To obtain reliable and accurate data, authors took all precautionary steps while acquiring the data from concern subjects. We took all possible efforts to establish a friendly relationship with the subjects; so that the workers do not hesitate to provide personal information and samples. Prior to interview, described the objectives of the study to all subjects in order to convince the main moto of the study and well explained that study not to diminish their livelihood and purely a research.

Biological sampling

Venous blood samples were collected carefully from the left arm cubital vein in two tubes with and without anticoagulant i.e. heparin, centrifuged at 800 g for 15 min to acquire plasma, washed with 0.9% NaCl, centrifuged at 800 g for 10 min and repeated thrice in order to get erythrocytes. Blood samples were kept at room temperature for 30 minutes, centrifuged at 1000 g for 10 minutes and supernatants were collected. All samples were aliquoted and kept at−80°C for further analysis.

De-proteinization

Plasma samples, 5 g of metaphosphoric acid (MPA) dissolved in 50 ml water to obtain the MPA reagent. An equivalent MPA reagent was added to the plasma samples, added to the vortex, subsequently the mixtures were incubated at room temperature for 5 minutes and centrifuged at 2000 g for 2 min. Supernatants collected and Erythrocytes were used for analysis of GR, GPx, CAT and SOD. Biochemical and GR, GPx, CAT and SOD parameters were analysed by using commercially available kits.

CAT activity

CAT was assessed colorimetric method by using commercially available kit at 540 nm, and the test results expressed in nmol/min/ml.

SOD activity

Activity levels of SOD was determined colorimetric method by using a commercially available kit at 450 nm and the results expressed in unit/ml. 1 unit of SOD is equal to enzyme that decomposed 50% of superoxide radical.

GPx activity

GPx activity measured in erythrocytesas described by Gunzler 1974[15] and estimated by following the manufacturer's procedure at 340 nm, at 1 min intervals for 5 periods, and the results were expressed in nmol/min/ml.

GR activity

The activity of GR was measured by a glutathione reductase assay kit, reduced GSSG by NADPH in the presence of GR at 340 nm, at 1 min intervals for 5 periods. The results expressed in nmol/min/ml.

Statistical Analysis

The data was tabulated and statistically evaluated by ANOVA with the help of SPSS version 24.0 package.

RESULTS AND DISCUSSION

The demographic characteristics of the subjects are shown in Table 1. Alcohol, and smoking habits and age were comparable among workers. Most of the workers had 16-20 years (34.5%), 6-10 (27.6%), 11-15 (20.7%) and 0-5 (17.2%) respectively. Usage of safety equipment claimed as follows gloves (17.2%), masks (27.6%), and safety goggles (31.0%). Study results evidenced that most of the workers were not using protection equipment for better safety. It is far away from the occupational health and safety measures. Most of the equipment's were made with fabric and low-quality material. Smoking habit of workers (37.9) and controls (66.7%) and was nearly 24-26 cigarettes per day.

Table 1: Demographic distribution of male quarry workers

Parameter	Control n = 24 (%)	Exposed group n = 29 (%)
Age		
17-27	4 (16.7)	8 (27.6)
28-38	6 (25.0)	6 (20.7)
39-49	9 (37.5)	13 (44.8)
50 and above	5 (20.8)	2 (6.9)
Smoking habit		
Smokers	16 (66.7)	11 (37.9)
Non smokers	8 (33.3)	18 (62.1)
Alcohol consumption		
Yes	0	16 (55.2)
No	24 (100)	13 (44.8)
Duration of exposure		
0-5		5 (17.2)
6-10		8 (27.6)
11-15		6 (20.7)
16-20		10 (34.5)
Usage of protective equipment		
Gloves		
Yes		5 (17.2)
No		16 (55.2)
Rarely		8 (27.6)
Mask		
Yes		8 (27.6)
No		11 (37.9)
Rarely		10 (34.5)
Safety goggles		
Yes		9 (31.0)
No		14 (48.3)
Rarely		6 (20.7)

Source: Field survey

Table 2: Levels of Catalase, SOD, GPx and GR of control and silica exposed workers

Parameter	Control n = 24	Silica dust exposed n = 29	ANOVA
Catalase (nmol/min/ml)	18.98 ± 4.21	14.91 ± 1.27	$p < 0.0001$
Superoxide dismutase (Unit/ml)	4091 ± 41.09	33.12 ± 6.45	$p < 0.0001$
glutathione peroxidase (nmol/min/ml)	47.91 ± 5.28	14.74 ± 8.40	$p < 0.0001$
glutathionereductase (nmol/min/ml)	21.56± 4.91	23.63 ± 9.65	$p < 0.0001$

Values are mean ± S.D. samples. Values in the parentheses are percent increase or decrease over control. Values are significantly different from control at $p < 0.0001$

The activity levels of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase in workers exposed to silica dust were presented in Table 2. The catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activity levels were significantly depleted ($p < 0.0001$) in exposed workers when compared with their respective control group.

Preventive antioxidants and chain breaking antioxidants are two primary classes. Preventive antioxidants inhibit oxidative actions which diminish cellular molecules. Chain breaking antioxidants destroys initiation of free radical chain reaction.

IARC (1997)[9] published and stated that studies of experimental and epidemiologic on various cancers in mammals, workers exposed to silica and presumed that there is significant evidence in humans to explore carcinogenicity, genotoxicity followed by inhalation of respirable silica from occupational sources. It is well known fact that ROS might also be responsible for inflammation and cancer[16]. Thus, it is vital to consider oxidative and immune stress biomarkers in occupational workers exposed to silica. An elevated level in oxidant stress biomarkers and depletion in the antioxidant parameters had been observed in workers exposed to silica. Our results in accordance with various studies, Abdelatty[17] reported that a depletion in activities of SOD, CAT, GSH, and IgG. Similarly, Sakhvidi[18] in ceramic workers in Iran and reported that the MDA levels in workers exposed to silica were higher than controls. *In vivo* studies demonstrated that silicosis and heavy metals[19-27] induces oxidative stress, which enhances generation of ROS and enhanced ROS associated with elevated levels of lipid peroxidation and oxidative enzymes activities[28-30].

We noted thatthe catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase activity levels were significantly depleted in exposed workers. No significant correlation was found in oxidative stress among alcohol use, smoking. Reddy 2020 demonstrated and reported; a significant alteration in white and red blood cell counts, *in silica* exposed workers. Data indicated that induction of oxidative stress and activation of immune system was associated with crystalline silica exposure. As a recommendation, to reduce silica exposure

and prevent toxic effects, specific ventilation practices, lowering the limit values of exposure as well as using suitable protective equipment, need to be incorporated. The data suggest that inducing oxidative stress and activation of the immune system is associated with crystalline silica exposure. Specific ventilation practices are needed in order to minimize exposure to silica and prevent toxic effects of silica in granite workers.

Catalase (CAT), an important antioxidant, contains iron as a prosthetic group, and tetrameric hemoprotein and present in peroxisomes; protects the cell from oxidative damage by converting hydrogen peroxide (H_2O_2) as H_2O and O_2. The levels of catalase activity vary in the patho-physiological conditions. Catalase originates via metabolism of endogenous or exogenous compounds. Since it inhibits the generation of reactive hydrogen peroxide from the cells, plays an important part in detoxification mechanisms and capable to secure cells from toxic damage[12-14]. Activity levels of GST could disrupt the superoxide ion radicals which make from xanthine oxidation to delay damages to cellular-organization at higher levels.

Antioxidant enzymes stimulate the primary cellular diminution process through oxidative stress. Studies have been reported on antioxidant enzyme and revealed that they protect cells against ROS released in different cell compartments[17,18]. CAT considered as free radical scavenging enzyme, and acts as body's secondary protection.

CONCLUSION

Data indicated that induction of oxidative stress and activation of immune system was associated with crystalline silica exposure. As a recommendation, to reduce silica exposure and prevent toxic effects, specific ventilation practices, lowering the limit values of exposure as well as using suitable protective equipment, need to be incorporated. We noted that the catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase activity levels were significantly depleted in exposed workers. The data suggest that silica dust inducing oxidative stress and activation of the immune system. Specific ventilation practices are needed in order to minimize exposure to silica and prevent toxic effects of silica in granite workers.

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