Activated Carbon Fabric Respiratory Mask reduces Blood Lead, Oxidative Stress and Improves the Antioxidant Status and Liver Functions in Unorganised Battery Workers

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ABSTRACT
Effect of use of Activated Carbon Fabric (ACF) mask on blood lead (PbB), lipid peroxide, erythrocytes-Superoxide Dismutase (SOD), Catalase, Ceruloplasmin, and liver functions tests of battery manufacturing workers (BMW) were studied. For this study, 36 male battery manufacturing workers, those who are regularly using ACF mask for two months were included. A blood sample was collected before and after using ACF masks from battery manufacturing workers and estimated the blood lead level, liver function tests, oxidative stress, and antioxidant status parameters by using the standard method. After two months use of ACF mask by BMW, the blood lead level (P<0.01, -15.76%), total serum bilirubin (P<0.05, -28.09%), alanine transaminase (P<0.05, -20.84%), lipid peroxide (P<0.001, -33.33%) were significantly decreased and erythrocytes-superoxide dismutase (P<0.05, 22.38%), catalase (P<0.05, 38.39%), ceruloplasmin (P<0.05, 13.13%) were significantly increased in battery manufacturing workers as compared to before using the mask. This study reflects the use of two months ACF mask of high lead-exposed workers are useful to decrease lead absorption, which results in decreases oxidative stress and improves the antioxidant status and prevents liver toxicity.

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INTRODUCTION

Lead is a ubiquitous, bluish-grey heavy metal, which naturally occurs in the earth’s crust (Atsdr, 2007). It is the first revealed and most extensively used metal. It has been stated widespread industrial use of lead and its emission as exhaust products have made a serious threat to human health (Juberg et al., 1997). Ninety-seven percent of lead is obtained from recycling the lead-acid batteries, and today maximum, it is used for the production of various lead-acid batteries (Atsdr, 2007). Occupational data reveals food, beverages, soil, and dust contaminated with lead are mainly absorbed from the gastrointestinal tract by the ingestion or inhalation. However, in the case of the occupational lead-exposed population, mainly the atmospheric air lead absorbed through inhalation (World Health Organization, 1995). Lead is distributed mainly in liver and kidneys, and maximum in the bone.

More than 90% lead accumulates into bone and half-life is 27 to 30 years. Lead is excreted through the urine, and tiny amounts are excreted through the faeces, sweat, hair, and nails (Atsdr, 2007).
Increased blood lead level damages all organs, i.e. liver, heart, kidneys, brain, and all the systems, the toxicity of lead mainly depends on the level and duration of lead exposure (Patil et al., 2007).

Elevated blood lead impairs the detoxification of xenobiotics. It alters tryptophan metabolism in the liver, which results in increase serotonin and hydroxyl indole acetic acid in the brain and disturbs the neurotransmitter functions. Lead also induces hepato-toxicity, leading to an alteration in liver enzymes (Kshirsagar et al., 2015). And such alterations were observed after chronic oral lead acetate administration to albino rats (Mahdy et al., 2012).

Lead induces the oxidative stress and alters the antioxidants such as glutathione peroxidase (GPx), glutathione-S-transferase, superoxide dismutase (SOD), and catalase (CAT), and a non-enzymatic molecule like glutathione (GSH) by covalent interaction with-sulphydryl (SH) groups of the antioxidant defence system, or by decreasing the zinc, copper, iron absorption. These are essential cofactor in the catalytic sites. (Flora et al., 2012; Ahamed and Siddiqui, 2007).

Activated Carbon Fabric (ACF) is a fibrous adsorbent. It is obtained from fibrous precursor by carbonisation and activation process. The ACF mask decreases the lead absorption of battery workers (Kuruville, 2013). In textile, dye, and metal processing industries, where lead is used, the ACFs are mainly used for the removal of lead from waste liquids by the adsorptive process (Basso et al., 2002; Betancur et al., 2009). The other toxic metals such as cadmium, copper, nickel, chromium, and zinc were also removed by using ACFs (Thakare et al., 2017).

Therefore, we were concerned to provide ACF masks to battery workers. We observed decrease absorption of the blood lead, improvement in liver functions, decrease oxidative stress and improvement of the antioxidant enzymes of battery workers from the unorganised sector.

MATERIALS AND METHODS

This study comprises thirty-six lead-exposed battery workers from Western Maharashtra, India. The age of study subjects was 20-45 in years, and they were non-alcoholic, non-smoker. Subjects of any significant/minor illnesses were excluded. The occupational, clinical data, socio-economic status and nutritional status were collected by interviews and using a pre-structured questionnaire. The written consent in local language was collected from all battery workers. Objectives of the study were informed to all these battery manufacturing workers. Protocol and institutional ethics committee approved this study (Ref. No. KIMSDU/IEC/03/2016, Dated 8/11/2016, Protocol No. 2016-2017/07). All the procedures of this study were followed as per the 1964 Helsinki declaration (Declaration of Helsinki, 1964).

All the battery workers were provided ACF mask and instructed to use this mask for two months. The regular use of an activated carbon fabric mask was confirmed through weekly telephonic communication to all these battery workers. The activated carbon fabric mask manufactured by Environment Care Products, Pvt. Ltd; Greater Noida. This full-face respirator mask is with activated carbon fibre. It acts as a barrier against chemical and biological impurities. It is an excellent absorbent of environmental toxin, pollen, atmospheric pollutant, and harmful gases. It can be used in almost all industries. The quality of mask approved according to the environmental standard.

The workers were guided to clean the masks (recharge). The ACF mask can be easily recharged by opening the valves and filter should be removed and dipped in boiling water for 5-10 minutes and dry under the sun. The authorities of Environ products Ltd. gave this instruction as a unique property of an ACF fabric is the possibility to “reactivate” the fabric when it has become saturated so that it can be used again. The durability of this mask is approximately six months to one year. This study was conducted, when the atmospheric temperature and humidity was low, i.e. in the winter session (October to December 2018). Study participated workers were using these masks continuously during their working hours.

The 10 ml blood was collected in a tube containing heparin and EDTA bulb for the estimation of biochemical parameters incorporated in the study. Standard methods are used to measure all biochemical parameters at our institute.

Blood lead level was estimated by using lead care II blood lead analyser (Magellan Diagnostics, USA). The principle is based on Anodic Stripping Voltammetry (ASV). RBC was lysed by using lead care treatment reagent, which releases lead from RBC. When a negative potential applied to the sensor, lead atoms accumulate on the test electrode. For releasing the lead ions, the potential was rapidly reversed, which generate the current, and it was directly proportional to the amount of lead in the blood sample (Ghanwat et al., 2016).

Kei Satoh method was used for the estimation of lipid peroxide (Kei, 1978). Marklund and Marklund
(1988) method was used to estimate the erythrocyte superoxide dismutase (SOD) activity (Nandi and Chatterjee, 1988). Catalase was estimated by the method of (Aebi, 1974). (Ravin, 1961) the method was used for plasma ceruloplasmin estimation.

A fully automated biochemistry analyser was used for estimations of liver function tests. AST and ALT were measured by the UV-kinetic method. For serum total proteins estimation Biuret method is used (Gornall et al., 1949). BCG method is used for the estimation of serum albumin (Doumas et al., 1997). Serum globulins and the A/G ratio were calculated by using total serum proteins and albumin values. Diazo method is used for the estimation of serum total bilirubin (Henry et al., 1974). Serum Alkaline Phosphatase (ALP) was measured by using a 2-Amino-2-Methyl-1-Propanol (AMP) (Varley, 1954). Statistical analysis of biochemical parameters was done by paired 't' test using Instat Graph Pad software. P-value was less than 0.05 was considered statistically significant.

RESULTS

There was a decrease in absorption of lead and decreases in serum total bilirubin, alanine transaminase with ACF masks in battery workers. And total serum proteins, albumin, albumin/globulin ratio, were increases significantly. However, globulin and aspartate transaminase levels were not altered. Oxidative stress was significantly decreased, and antioxidants like superoxide dismutase, catalase, ceruloplasmin were significantly increased as compared to without using activated carbon fabric masks. Figure 1

DISCUSSION

Blood lead levels (p<0.01, -15.76%) was significantly decreased subsequently using ACF masks, which indicates that the decrease absorption of lead (Table 1 & Figure 1). Lead enters the body through inhalation and ingestion of contaminated food at the site of the workplace. In this study mainly unorganised sector battery manufacturing workers were enrolled and they were involved in smelting, recycling, and plating of lead batteries and these workers were highly exposed to lead, which results in increased blood lead levels. Therefore, we provided activated carbon fabric masks to these workers and requested all these workers to use regularly this special mask for two months. We communicated these workers and owners of industries telephonically every week to ensure that all workers using this mask regularly. We have found that the consistent use of ACF mask is beneficial to reduce the blood lead levels of battery workers.

Battery workers had severe symptoms of lead toxicity, were nausea, loss of appetite, constipation, intermittent abdominal pain, diarrhoea, and joint pain. Acute and chronic symptoms of lead toxicity observed in battery workers without using activated carbon mask were depicted in (Table 2). However, there was no significant decrease in acute and clinical symptoms, even after using the ACF mask, which may be due to a high concentration of lead in the blood, soft tissues, and bones. Therefore, it is essential that symptoms of lead can be recognised at an early exposure period to minimise the toxic effects. Acute and chronic clinical symptoms are reduced by decreasing the blood lead level. Therefore, to reduce lead exposure, precautionary measures can be used. Among these, some are like using special masks, apron, goggles, and even rotating the place of workers from high lead exposure to low lead exposure.

With ACF mask after two months, alanine transaminase activity (P<0.05, -20.84%) significantly reduced. However, aspartate transaminase (~7.06%) was not statically significantly altered as compared to without using masks in battery workers. In our earlier study, increased AST and ALT activities were observed in battery workers as compared to control subjects, which indicate that increased blood lead level damages the liver function and similar findings were also reported in earlier studies (World Health Organization, 1995; Patil et al., 2007; Mazumdar and Goswami, 2014). Therefore, the regular use of ACF mask may reduce the adverse effects of lead on the liver.

Serum total proteins (P<0.01, 1.23 %), albumins (P<0.001, 2.66%) and albumins/globulins ratio (P<0.01, 2.29%) were slightly increased; however, serum globulins were not altered significantly with using ACF mask in battery workers. Decreased serum total proteins and albumin levels as compared to control in battery manufacturing workers were well documented in previous studies (World Health Organization, 1995; Patil et al., 2007; Mazumdar and Goswami, 2014). Lead-intoxicated experimental animal studies have reported decreased protein synthesis due to high blood lead levels. The reduction of serum total proteins and albumins mainly due to decreased synthesis of albumin and other proteins at high blood lead levels were observed in earlier studies (Kshirsagar et al., 2015). We observe continuous use of the ACF mask in high lead-exposed workers is beneficial to prevent the damage of parenchymal tissue of the liver, which synthesises albumins and proteins.

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Figure 1: Percentage change of Blood lead levels, Liver function tests, lipid peroxide, SOD, Catalase, ceruloplasmin with respect to before using activated carbon fabric respiratory mask

Table 1: Blood Lead Levels, Liver Function Tests, lipid peroxide, SOD, Catalase, Ceruloplasmin of Battery Workers, without & with using ACF Masks

| Sr. No. | Lab investigations                     | Without ACF Masks (N = 36) | With ACF Masks (N=36) |
|---------|----------------------------------------|----------------------------|-----------------------|
| A       | Blood Lead Level (µg/dl)               | 88.31 ± 18.16 (47.6-130.20) | 74.39 ± 20.16** (36.7-126.98) |
| B       | Liver Function Tests                   |                            |                       |
| 1       | AST (U/L)                              | 24.33 ± 11.51 (12.70-71.7) | 22.61 ± 11.07**(10.80-68.90) |
| 2       | ALT (U/L)                              | 30.80 ± 13.37 (12.60-64.80) | 24.38 ± 12.23*(9.40-53.20) |
| 3       | Total Proteins (gm/dl)                 | 7.29 ± 0.15 (6.89-7.49)    | 7.38 ± 0.12**(7.14-7.57) |
| 4       | Albumin (gm/dl)                        | 4.12 ± 0.09 (3.90-4.24)    | 4.23 ± 0.07*** (4.06-4.34) |
| 5       | Globulin (gm/dl)                       | 3.16 ± 0.12 (2.91-3.47)    | 3.18 ± 0.10 (2.91-3.36)   |
| 6       | Albumin/Globulin ratio                 | 1.31 ± 0.06 (1.12-1.43)    | 1.34 ± 0.05** (1.24-1.45) |
| 7       | Bilirubin (mg/dl)                      | 0.89 ± 0.04 (0.27-2.9)     | 0.64 ± 0.40 (0.21-2.0)    |
| 8       | ALP (U/L)                              | 84.06 ± 17.01 (46-125)     | 72.42 ± 15.89** (47-98)   |
| C       | Serum Lipid Peroxide (nmol/ml)         | 2.22 ± 0.45 (1.2-3.08)     | 1.48 ± 0.283*** (0.75-2.11) |
| D       | RBC-Superoxide Dismutase (unit/ml of haemolysate) | 7.82 ± 2.71 (1.74 - 13.04) | 9.57 ± 3.34* (1.74-16.55) |
| E       | RBC-Catalase (mM/H2O2 decom/mgHb/min)  | 9.90 ± 6.39 (4.23-25.35)   | 13.75 ± 8.35* (4.23-46.48) |
| F       | Plasma Ceruloplasmin (mg/dl)           | 41.06 ± 11.15 (20.10-58.80) | 46.45 ± 10.67* (23.70-72.70) |

AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase. Figures indicate mean ± SD values and those in parenthesis are minima to a maximum range of values of the specific group; *P<0.05, **P<0.01, ***P<0.001, #- non-significant
Table 2: Mild and Severe Symptoms of Battery Workers

| Sr. No. | Symptoms                                      | Percentage (%) |
|---------|-----------------------------------------------|----------------|
| A       | Mild symptom (Adult PbB level> 60 mg/dl)      |                |
| 1       | Muscle Pains                                 | 42.8           |
| 2       | Prickly itchy feeling                        | 38.0           |
| 3       | Mild fatigue                                 | 33.3           |
| 4       | Aggressiveness                               | 50.0           |
| 5       | Irritability                                 | 47.6           |
| 6       | Lethargy                                     | 50.0           |
| 7       | Abdominal discomfort                         | 40.4           |
| B       | Severe symptoms (PbB level -70-80 mg/dl)     |                |
| 1       | Joint pain                                   | 47.6           |
| 2       | General fatigue                              | 38.0           |
| 3       | Poor concentration                           | 26.2           |
| 4       | Tremor                                       | 33.3           |
| 5       | Headache                                     | 45.2           |
| 6       | Abdominal pain                               | 40.5           |
| 7       | Constipation                                 | 35.7           |

Serum total bilirubin (P<0.05, -28.08%) was significantly decreased by using ACF mask in battery workers. Increased blood lead level increases the rate of red blood cell hemolysis and increases serum bilirubin levels were reported in several earlier studies (Tishkoff et al., 1958). Jaundice and raised serum bilirubin were also reported in several cases of lead poisoning. Maugeri and Lavoro, (1940) reported in industrial lead poisoning increased serum bilirubin and excretion of stercobiligen and urobiligen. It concluded that lead anaemia was the hemolytic type (Henderson, 1952). We observe a decrease in serum bilirubin after continuous use of ACF mask indicates it may be due to decrease blood lead level, which might reduce the accumulation of lead in erythrocytes and decreases the RBC's lysis.

Serum lipid peroxide is the indicator of oxidative stress (P<0.001, -33.33%) significantly decreased and antioxidants such as erythrocyte superoxide dismutase (P<0.05, 22.37%), catalase (P<0.05, 38.88%) and ceruloplasmin (P<0.05,13.12%) were increased significantly with ACF mask in battery workers, which indicates the generation of reactive oxygen species or free radicals are decreases, which results in improving the antioxidant status parameters might be due to decreased blood lead level after regular using two months of ACF mask.

Several works have described increased blood lead level induces the oxidative stress due to the various reasons, i.e. Interaction of lead with oxy-haemoglobin, accumulation of δ-ALA in RBC, increased δ-ALA may undergo enolisation and antioxidation at pH 7.0–8.0, resulting in more generation of superoxide anion (Ahamed and Siddiqui, 2007). Coupled oxidation of δ-ALA / oxy-haemoglobin results in the generation of reactive oxygen species (Ahamed and Siddiqui, 2007). Accumulation of lead in RBC altered the membrane structure, functions, enzyme activity and proteins composition, which increases the osmotic and mechanical susceptibility of RBC's finally leads to more oxidative damage (Donaldson and Knowles, 1993; Raghavan et al., 1981). Therefore, the reduction of blood lead level by using continuous ACF mask, apron, and goggles are the solutions to reduce the oxidative stress.

Erythrocyte-SOD was significantly reduced in high lead-exposed workers such as battery manufacturing workers might be due to more superoxide radical generation and low level of serum copper (Atsdr, 2007; Juberg et al., 1997). Lead reduces the absorption of copper from the gastrointestinal tract since lead is divalent, and due to copper deficiency, the RBC-SOD activity decreases since SOD is a Zn-Cu-containing enzyme.

Erythrocyte catalase was significantly reduced in battery manufacturing workers, might be due to more generation of free radicals which decreases heme level and decreases iron absorption at the GIT level. The collective effect of these mechanisms decreases the heme pool. Thus, it is evident that the lead is responsible for the decrease in erythrocytes-catalase activity since it is a heme-
containing enzyme.

Our study shows that regular use of ACF mask in high lead-exposed workers is beneficial to reduce oxidative stress and improve antioxidant status.

CONCLUSIONS

Regular use of activated carbon fabric masks in battery workers reduce lead absorption, which resulted in a low level of blood lead, lipid peroxide and improved the antioxidants. ACF mask also prevents liver toxicity. It is also essential that the owners of battery manufacturing industries should provide activated carbon fabric mask to all workers. They should also monitor regularly to these workers for using this mask.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Consent to participate

Informed consent was obtained from all individual participants included in the study.

Ethics approval

The study protocol was approved by the protocol and institutional ethics committees (Ref. No. KIMS DU/IEC/03/2016, Dated 8/11/2016, Protocol No. 2016-2017/07) before the enrolment of study participants. And all the procedures of this study were followed as per the 1964 Helsinki declaration.

Authors Contributions

The following authors participated for manuscript preparation,

1. Dr. (Mrs). Jyotsna A. Patil- Design of the work, acquisition, analysis, drafting the work, revising it critically for important intellectual content.
2. Dr. (Mrs) Mandakini S. Kshirsagar- Design of the work, acquisition, analysis, drafting the work.
3. Dr Arun J. Patil- conception, interpretation of data for the work, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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