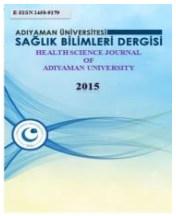
Adıyaman Üni. Sağlık Bilimleri Derg, 2019; 5(2);1500-1510

ADIYAMAN ÜNİVERSİTESİ SAĞLIK BİLİMLERİ DERGİSİ
E-ISSN 2458-9179
dergipark.gov.tr/adiyamansaglik



Araştırma/Research

Evaluation of lung toxicity in rats kept in coal mine ambience by in vivo respiration records: An experimental study

Saliha Ayşenur Çam [1], Seyfullah Oktay Arslan [1]

[2] [1]Pharmacology Department, Medical Faculty, Ankara Yildirim Beyazit University, Ankara, Turkey.

Abstract

OBJECTIVE: Mine workers exposed to dusts or toxic gasses in occupational atmospheric conditions. Pneumoconiosis and other lung diseases are charecterized by the pathogenesis of coal dust-caused pulmonary toxicity associated with reactive oxygen species (ROS).

MATERIALS AND METHODS: This study was conducted to investigate the respiration failures and fibrosis in rats after being exposed to coal dust and gases in mine atmosphere. Another aim was to study the therapeutic effect of erdosteine as antioxidant therapy. Rats were exposed to mine ambience for four week, and then they were breathed in the clean air for four week. The respiratory functions of rats were recorded once a week for eight week, as in vivo. The fibrosis of lung tissue levels, the oxidant/antioxidant status, and cytokines of inflammation in bronchoalveolar lavage fluids (BALFs) were evaluated, at the end of the processes.

RESULTS: We observed to be the beginning of respiratory abnormalities in animals exposed to coal dust in second week. The end of fourth week, there were the increase of respiratory frequency and along with the decrease of respiratory depth. The respiratory failures were not improved in clean ambience, moreover apnea were appearance in the end of six week (the second week of clean air). Deaths were 28% in animals. Erdosteine administer to rats could not fully abolished to the pulmonary toxicity, however could able to hold to toxicity, and also there were not dies in rats administered to erdosteine. Coal dust exposure was resulted in fibrosis with higher hydroxyproline (HP) levels, cytokine inflammation with higher interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) levels, and lipid peroxidation with an increased malondialdehyde (MDA) levels, according to the healthy. A dramatically run out of endogen antidote sulfide pools (GSH), and an increased MPO activity were dedected in the mine dusts and gasses exposure group, according to the healthy animals. High biochemical index of toxicity were partly balanced by erdosteine.

CONCLUSIONS: Our experimental findings support the hypothesis that ROS is induced coal workers' pneumoconiosis. Re-oxygenation cannot be getting it together to reverse the pulmonary toxicity. On the top of it, it can make its pathogenesis further exaggerating and even worse. On this account we heartily speculate that re-oxygenation should be by steps in mine workers. In addition the antioxidant therapy may be partly a choice be able to tolerate the coal dust-induced lung toxicity of mine workers.

Key words: Respiratory functions, antioxidant therapy, re-oxygenation, oxidative stress, coal mine ambience.

Yazışmadan Sorumlu Yazar

Seyfullah Oktay Arslan
Pharmacology Department, Medical Faculty, Ankara Yildirim Beyazit University, Bilkent, Ankara, Turkey
Tel : +90 312 906 20 81
Email: soarslan@gmail.com

Doi: 10.30569.adiyamansaglik.596944

Geliş Tarihi:	**25.07.2019**
Kabul Tarihi:	**08.08.2019**

Kömür madeni ortamında tutulan sıçanlarda akciğer toksisitesinin in vivo solunum kayıtları ile değerlendirilmesi: Deneysel bir çalışma

Amaç: Maden işçileri çalışma ortamı koşullarında tozlara veya zehirli gazlara maruz kalmaktadır. Pnömokonyoz ve diğer akciğer hastalıkları, kömür tozu kaynaklı pulmoner toksisitenin reaktif oksijen türleriyle (ROS) ilişkili patogenezi ile karakterizedir.

Yöntem: Bu çalışma, maden atmosferinde kömür tozu ve gazlarına maruz kaldıktan sonra sıçanlarda solunum yetersizliği ve akciğer fibrozunu araştırmak için yapıldı. Diğer bir amaç, erdosteinin antioksidan tedavi olarak terapötik etkisini araştırmaktı. Sıçanlar, dört hafta boyunca maden ortamına maruz bırakıldı ve daha sonra dört hafta boyunca temiz havada kaldılar. Sıçanların solunum fonksiyonları, in vivo olarak, sekiz hafta boyunca haftada bir kez kaydedildi. İşlem sonunda, akciğer dokusu seviyelerinin fibrozisi, oksidan / antioksidan durumu ve bronkoalveoler lavaj sıvılarında (BALF) inflamasyonun sitokinleri değerlendirildi.

Bulgular: İkinci haftada kömür tozuna maruz kalan hayvanlarda solunum anormalliklerinin başladığı gözlendi. Dördüncü haftanın sonunda, solunum sıklığının artması ve solunum derinliğinin azalması vardı. Solunum yetersizlikleri temiz ortamda iyileşmedi, ayrıca altı haftanın sonunda (temiz havanın ikinci haftasında) apne ortaya çıktı. Hayvanlarda ölümler % 28 idi. Ratlara uygulanan Erdostein, akciğer toksisitesini tamamen ortadan kaldıramadı, ancak toksisitedeki şiddeti azalttı ve ayrıca erdostein uygulanan sıçanlarda ölen yoktu. Kömür tozuna maruz kalma, yüksek hidroksiprolin (HP) seviyelerine sahip fibrozis, yüksek interlökin-6 (IL-6) ve tümör nekroz faktörü-alfa (TNF-α) seviyelerinde sitokin iltihabı ve artmış malondialdehit (MDA) ile lipit peroksidasyonuyla sonuçlandı. Sağlıklı hayvanlara göre, endojen sülfit havuzları (GSH) dramatik bir şekilde tükendi ve artmış bir MPO aktivitesi tespit edildi. Yüksek biyokimyasal toksisite indeksi kısmen erdostein ile dengelenmiştir.

Sonuç: Deneysel bulgularımız, ROS'un kömür işçilerinin pnömokonyozu oluşturduğu hipotezini desteklemektedir. Akciğer toksisitesini tersine çevirmek için yeniden oksijenasyon yarar sağlamaz. Üstelik, patogenezini daha da kötüleştiriyor. Bu nedenle, yeniden oksijenasyonun maden işçilerinde adım adım olması gerektiğini kuvvetle düşünüyoruz. Ek olarak, antioksidan tedavi, kısmen, maden işçilerinin kömür tozu kaynaklı akciğer toksisitesini tolere edebilmesi için bir seçenek olabilir.

Anahtar Kelimeler: Solunum fonksiyonları, antioksidan tedavi, re-oksijenasyon, oksidatif stres, kömür madeni ambiyansı.

Introduction

The inhalation of coal dusts by mine workers cause very serious, progressive and persistent lung disorders, including irreversible pneumoconiosis or fibrosis, chronic bronchitis, pulmonary failure, and emphysema (1). Reactive species (ROS or NOS) have been showed a critical role in the development of coal dust-induced pulmonary toxicity. Coal dust can stimulate the macrophage and provocation of pro-inflammatory cytokines and disrupt the oxidant/antioxidant balance. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are been in migrated polymorphonuclear cells as provocative factors in inflammation of lung tissue. The extend ROS amounts and cytokines is produced by activated macrophages (2-4). Both experimental and clinical researches have been produced on the mechanisms of pathogenesis of pulmonary toxicity caused by coal dusts. Studies have been focused on oxidant damage and

antioxidant protection (5,6). Erdosteine has the sulfhydryl groups, for that reason it can has a ROS scavenger and/or antioxidant properties (7).

The aim of present research was to reveal: i) the deleterious results of coal mine ambience and dusts on pulmonary toxicity; ii)a mechanism of inflammatory processes via oxidant damage; iii) a mechanism of inflammatory processes via production of pro-inflammatory cytokines; and iv)the unpredictable effects of re-oxygenation or antioxidant therapy in rats. For all, these processes were conducted: 1) in vivo respiration records, 2) lung collagen deposition, HP levels in lung tissue; 3) lipid peroxidation, MDA levels; 4) polymorphonuclear leukocyte accumulation; MPO activity; 5)endogen antioxidant sulphide pools, GSH levels; and 6)pro-inflammatory cytokines, the IL-6 and TNF-α levels of BALFs.

Materials and Methods

Animals, experimental design, coal mine ambient conditions

In study, after we got approval by the ethics committee of Bulent Ecevit, Wistar rats, weighing 250-300 g, was used. Rat chow and tap water were given as *ad libitum*. While control animals were kept comfortable in the pharmacology laboratory for 8 weeks, experiment groups, for 4 weeks, were kept in the ambience of Kozlu Coal Mine of Zonguldak (-500 m). A subgroup was administered oral erdosteine, with everyday 10 mg/kg dose body weight, during the period of second 4 weeks.

The chemical circumstance of Kozlu mines was like that; 55% carbon, 26% volatile compounds, 11% ash, 8% moist. The chemicals of ash were including 45.7% of SiO_2, 26.4% of Al_2O_3, 8.1% of Fe_2O_3, and 6.8% of CaO. Other chemicals such as MgO, K_2O, Na_2O, TiO, P_2O_5 SO_3 were been detected totally 12.7%, and the others 1.3%. The diameters and average concentration of dusts in mine ambience were 1.17mg/m^3 and 0.5-5μm respectively. The gas measurements in experiment period were approximately 0.5% - 0.6% of CH_4, 0.05% of CO_2, 20.5% of O_2 and 1 ppm of CO. The all numbers of ambience concentration or ratio were given by Kozlu Coal Establishment.

On day 28th rats were received to the clean air from the atmosphere of coal mine. On day 56th they were sacrificed under anaesthesia for HP and BALF analysis.

Respiratory records

The respiratory functions of animals, for three groups, were recorded as in vivo a time of every week (totally 8 records), by Biopac record system.

Bronchoalveolar lavage fluid collection

The rats anesthetized using Na thiopental at a dose of 70 mg/kg (i.p.) were sacrificed on day 56 of the initiation of therapy. The thorax was gently opened with a median incision and the trachea was cannulated with a useful catheter that joined the 10 mL syringe. The fluids of bronchoalveolar lavage were collected in 5 mL portions with a total volume of 25 mL of sterile saline and gentle massage of the lung lobes. Total BALFs were centrifuged at 300 g for 10 minutes at 4°C. The supernatant obtained was used for biochemical studies.

Biochemical assays

The lavage fluid was analyzed for glutathione (GSH) following the method previously described by Adams et al.(8) and the MDA methabolite of LPO as is described by Ohkawa et al.(9) IL-6 and TNF-α level in BAL fluids were measured by ELISA method according to the manufacturer's kits using guideline for users. MPO activity in BALFs, an index of polymorphonuclear cell accumulation, was detected by the change in absorbance at 460 nm using o-dianisidinedihydrochloride and hydrogen peroxide (10).

After BALF was performed, the lobes of lung were removed; washing in ice-cold saline was applied, and soon after rapidly transferred to storage at -20°C for hydroxyproline detection. Frozen lung lobes were dissolved and homogenized in saline using a Polytron. In the next step, homogenates were moved to tubes and stored at -20°C until tested. The hydroxyproline content of lung tissue was determined spectrophotometrically. The procedue was previously explained by Woessner (11).

Statistical analysis

Statistical analyses of the data were done using SPSS software. Differences in founded values among groups were analyzed by one-way analysis of variance (ANOVA) and Bonferroni posthoc test for multiple comparisons was followed. Data were showed as mean ± standard deviation. If *p* values less than 0.05 were considered significant.

Results

Toxicity and fibrosis

Deaths were 28% in animals exposed mine ambience with dust and gasses, without antioxidant treatment, while there was no any death in erdosteine treated rats (table 1). Body weight was significantly decreased in animals exposed mine ambience; however erdosteine treatment was not reversed the weight loss (table 1).

Mine air conditions clearly caused a significant increased in the content of lung collagen detected as HP in tissue homogenates as compared to the controls. HP is the sign of pulmonary fibrosis of lung tissue. The detection of higher HP amounts in coal mine housed rats compared to controls was statistically significant (table 1). Erdosteine administered to rats could not abolish pulmonary toxicity, however it was able to hold to toxicity, and also there were no dies in rats administered to erdosteine.

Respiratory records and numbers

We observed to be beginning the respiratory abnormalities in animals to expose to coal dust and gasses in second week. The end of fourth week, there were the increase of respiratory frequency and along with the decrease of respiratory depth. The respiratory failures were not improved in clean ambience; moreover apneas were appearance at the end of six week (at the second week of clean air) (figure 1).

As seen table 2, there was approximately quadruple increase at respiratory numbers of mine ambience exposed rats per minute, when compared to control animals. Interestingly, after the clean air administer to rats, respiratory numbers were balanced to normal position in first and second weeks, however the decreasing of respiratory numbers was continue dramatically.

Lipid peroxidation, oxidant and antioxidant parameters in BALFs

The coal mine dust and gasses exposure resulted in a significant increase in the lipid peroxidation product, MDA levels. Biochemical parameters analyzed in BALFs and tissue samples are presented in the Table 1. It was found that MDA a level, oxidative marker was increased in housed animals. Antioxidant GSH amounts were dramatically run out compared to control.

Myeloperoxidase activity in BALFs

As seen table 1, myeloperoxidase activity and the migration of neutrophil and macrophage were higher than controls. Erdosteine administration significantly decreased high MPO activity.

Pro-inflammatory cytokines responses in BALFs

When compared to controls, the levels of Pro-inflammatory cytokines (IL-6, TNF-α) were significantly found to be higher. These parameters were showed to be partly reversed in Erdosteine-treated group but not the control rats. Erdosteine administration made no changes in the cytokine (IL-6, TNF-α) levels (table 1).

Table 1. Biochemical values analyzed in lung tissue or BALFs samples.

Groups **Parameters**	**Control** **(n:7)**	**Untreated** **(n:7)**	**Erdo** **(n:7)**
Survival	7 at 7	5 at 7	7 at 7
Body weight (g)	281 ± 13	238 ± 8a	247± 10 ns
HP (mg/g dried tissue)	1.04 ± 0.19	1.62 ± 0.4a	1.48 ± 0.35ns
MDA (nmol/ml)	0.150 ± 0.2	0.592 ± 0.5a	0.418 ± 0.2b
GSH (µmol/ml)	0.83 ± 0.10	0.18 ± 0.06	0.27 ± 0.10
MPO (mU/ml)	1.50 ± 14	12.4 ± 15a	6.2 ± 13b
IL-6 (ng/ml)	12.1 ± 4.1	11.8 ± 6.3	12.4 ± 4.9 ns
TNF-α (ng/ml)	11.1 ± 4.6	24.8 ± 2.6a	21.3 ± 4.0 ns

[a]$p < 0.05$; Untreated group in comparison with control group. [b]$p < 0.05$; Comparison of erdosteine treated and untreated groups. [ns]Non-significant with the comparison of erdosteine treated and untreated groups.

Table 2. Respiratory numbers of the rats affected by coal mine ambience.

Weeks	**Respiratory Numbers**			
	Control (n=7)	**Coal Ambience (n=14)**	**Clean Air (n=5-7)**	**Clean Air + Erdo (n=7)**
1st	80 ±8	85 ± 12	-	-
2nd	85 ±10	180 ± 25[a]	-	-
3rd	84 ± 12	210 ± 19[a]	-	-
4th	90 ± 12	280 ± 30[a]	-	-
5th	82 ± 10	-	120 ± 15[a]	130 ± 20[a]
6th	88 ± 10	-	60 ± 14	72 ± 15
7th	84 ± 8	-	55 ± 17	80 ± 17
8th	90 ± 11	-	30 ± 8[a]	54 ± 14[ab]

[a]$p < 0.05$; Untreated group in comparison with control group . [b]$p < 0.05$; Comparison of erdosteine treated and untreated groups. [ns]Non-significant with the comparison of erdosteine treated and untreated groups.

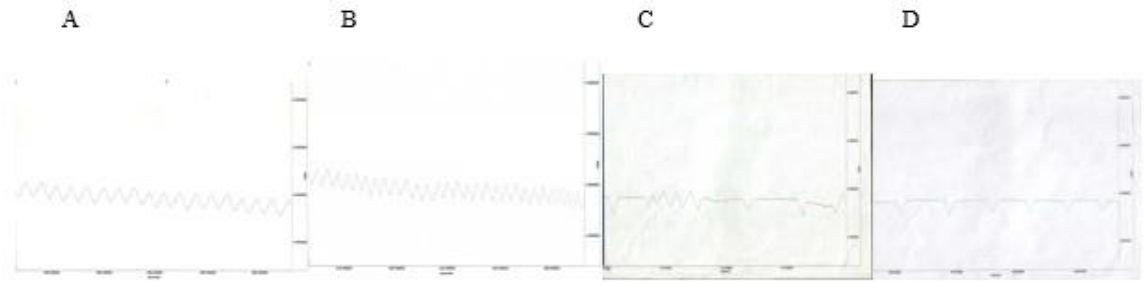


Figure 1. Respiratory records in rats. Respiratory frequences, Abnormalities and Apneas in clean air after Coal Mine Ambiance. A: control; B: at 2nd week; C: at 6th or 7th or 8th weeks; D: at 7th or 8th weeks.

Discussion

Our experimental findings show that the exposure to coal dusts and other air pollutants are caused early pulmonary reactions, including acute toxicity, respiratory abnormalities, disrupted oxidant/antioxidant status, exaggerated inflammation, and fibrosis in coal mine atmosphere.

In present research, rats were housed in coal mining that natural ambience for workers. In this way we were able to clinical scenario and interpretation from animal experiments. Our research differs from other fictitiously standardized animal studies in this respect. Pneumoconiosis has been reported in coal workers, mining where animals feed for present research. The prevalence of pneumoconiosis ranged between 1.23-6.23 percent in Zonguldak (12).

Firstly the results of our investigation on subacute appearance showed that pulmonary toxicity caused by coal dusts, gasses, and ambience is progressive, persistent, and irreversible in rats housed in exact coal mining. Although animals were exposed to fresh air after a month of exposure, weight losses and deaths were seen in the following days (table 1). This subacute toxicity may be related to failure lung functions in consequence of pulmonary fibrosis and pneumoconiosis (12,13). Secondly the results of our investigation showed on failure of lung functions that respiratory abnormalities is starting after 14 days in rats housed in exact coal mining (Figure 1). These symptoms may be related to first phase inflammatory of provocation due to dusts and gasses in coal mining. Accepted hypothesize that recurrent passages of acute lung injury caused by coal mine particles could finally lead to fibrosis, and eventually, failure of respiratory functions (13).

Thirdly the results of our investigation showed on oxidant/antioxidant system that defensive balance is disrupted in pulmonary cells in BALFs. The findings show, that following 28 days of exposure particles and gasses in coal mine and plus 28 day re-oxygenagation with clean air

circumstance, there was an increase in the MDA levels of BALFs cells (Table 1). The lipid peroxidation can be caused cellular damage via metal contact reactions or chelating and radical generation. This toxic mechanism, with earlier reports, has been demonstrated the both in laboratory conditions with mine dust models and in occupational clinic examine of coal workers (14-18).

Studies have demonstrated the pathogenetic mechanisms of mine dust-caused fibrosis and progressive lung diseases, associated with oxidative damage, inflammation, and provocation of cytokines (17-19). Coal workers can easily exposed to via inhalation of toxic coal dusts in the workplace. Actually, papers said that toxic effects in pulmonary tissues are attributed to ROS produced due to exposure to coal dusts. The production of ROS by the oxidation of macrophages and neutrophils occurs during inflammation (2).

This research discussed the role of coal mine ambience as a mark of oxidative damage in the pulmonary damage. It is suggest that coal dusts and/or gasses exposure evoke to chronic pulmonary oxidative damage. Thus the supplementation of antioxidants could have prophylactic and therapeutic value. For that reason, erdosteine as an antioxidant and sulfydryle structure was administered orally during 28 days at the end of exposure of 28 days. However its prophylactic effects were observed not satisfactory, just partly and low with supported several results.

It is known that coal dust is initiating the process of pulmonary fibrosis by triggering rapid inflammatory reactions in the first step. When the number of macrophages increases, inflammatory cells in injured tissue (i.e.leucocytes, fibroblasts, etc.) are induced leading to the formation of provocative cytokines (e.g. interleukins, TNF-a) and proteins (e.g. fibronectin) (14, 15). ROS were shown to be the main toxic components that enhance inflammation caused by mine dusts (15). Oxidant injury results when the damage caused by reactive free radicals overwhelmes the antioxidant defense mechanisms.

In present research, we have shown that coal mine ambience exposure resulted in a substantial depletion in GSH levels of BALFs, while erdosteine administration cannot reverse GSH depletion. All body tissues, including lung cells, have Glutathione as a ubiquitous intracellular thiol reservoir. GSH protects cellular integrity by maintaining a reducing environment. GSH is also involved in detoxification of various xenobiotics and synthesis of proteins, nucleic acids, and leucotrienes (16-18). GSH defends the lung tissue against oxidative injury caused by

various xneobiotic, organic and anorganic pulmonary toxicants. The depletion of GSH in the lung is related to a higher risk of pulmonary tissue injury (8,16).

Earlier reports remark that ROS play a main mechanism role in coal mine cytotoxicity. ROS are able tocause disruption of lipids, protein and DNA of living cells. The presence of an unpaired electron in their outer orbital of the free radicals makes them highly reactive. The superoxide anion radical (O2) is formed when a single electron is acquired by O2. The superoxide anion radical (O2) can then be reduced to hydrogen peroxide (H2O2) by the antioxidant enzyme SOD. Hydrogen peroxide may then be reduced to the hydroxyl radicals, which are the most toxic of the oxygen-based radicals (19).

Fourthly the results of our investigation showed on cytokines that IL-6 and TNF-α production is clearly increased with pro-inflammatory role, in BALFs. TNF-α and IL-6 are a pro-inflammatory cytokines, important in the onset of inflammation, development, and progression of pulmonary fibrosis, and associated with the progression of pneumoconiosis in inflammation caused by mine dusts. Papers indicate that the high levels of pro-inflammatory cytokines such as TNF-α and others is an increased risk of pneumoconiosis progression in mine workers, even in retired years (20-23).

It is well known that oxygen reperfusion of tissue is to aggravate the injury of cells (24). On this account re-oxygenation should be by steps in mine workers. In addition the processes of antioxidant therapy may be partially useful in the coal dust-caused pulmonary toxicity of coal workers. Indeed, one speculation of this report is that it focuses on the view the toxic effects of oxygen in retirement years are at least as serious as occupational exposure in mine workers.

CONCLUSIONS: Our experimental findings support the hypothesis that ROS is induced coal workers' pneumoconiosis. Re-oxygenation cannot be getting it together to reverse the pulmonary toxicity. On the top of it, it can make its pathogenesis further exaggerating and even worse. On this account we heartily speculate that re-oxygenation should be by steps in mine workers. In addition the antioxidant therapy may be partly a choice be able to tolerate the coal dust-induced lung toxicity of mine workers. A speculate that: Coal workers should not try more than 10 years in mine. CWP develops on average at the end of 13 years of underground coal mine exposure[12]. As a result of this study, if this experimental work could adapted to the clinic, we declare that we have a clear speculation: Re-oxygenation should be by steps in mine workers, when they especially in the beginning of retirement. So that it reduced the risk of oxygen toxicity.

Financial support
Nil.

Conflict of interest
Nothing.

References

1. Zhang Q, Huang X. Induction of ferritin and lipid peroxidation by coal samples with different prevalence of coal workers' pneumoconiosis: role of iron in the coals. Am. J. Ind. Med. 2002, 42: 171–179.

2. Pinho RA, Bonatto F, Andrades M, Frota ML Jr, Ritter C, Klamt F, et al. Lung oxidative response after acute coal dust exposure. Environ Res. 2004 Nov;96(3):290-7.

3. Nikula KJ, Vallyathan V, Green FH, Hahn FF. Influence of exposure concentration or dose on the distribution of particulate material in rat and human lungs. Environ Health Perspect. 2001 Apr;109(4):311-8.

4. Tao F, Gonzalez-Flecha B, Kobzik L: Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic Biol Med 35(4): 327-340, 2003

5. Vanhee D, Gosset P, Boitelle A, Wallaert B, Tonnel AB: Cytokines and cytokine network in silicosis and coal workers' pneumoconiosis. Eur Respir J 8(5): 834-842, 1995

6. Pinho RA, Silveira PC, Silva LA, Luiz Streck E, Dal-Pizzol F, F Moreira JC. N-acetylcysteine and deferoxamine reduce pulmonary oxidative stress and inflammation in rats after coal dust exposure. Environ Res. 2005 Nov;99(3):355-60.

7. Braga PC, Dal Sasso M, Zuccotti T: Assesment of the antioxidant activity of the SH metabolite I of erdosteine on human neutrophil oxidative bursts. Drug Res 50(8): 739-746, 2000.

8. Adams JD, Lauterburg BH, Mitchell JR. Plasma glutathione and glutathione disufide in the rat: regulation and response to oxidative stress. J. Pharmacol. Exp. Ther. 1983 227:749±754.

9. Ohkawa N, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979, 95:351±358.

10. Parlar A, Arslan SO, Doğan MF, Çam SA, Yalçin A, Elibol E, et al. The exogenous administration of CB2 specific agonist, GW405833, inhibits inflammation by reducing cytokine production and oxidative stress. Exp Ther Med. 2018 Dec;16(6):4900-4908.

11. Woessner JF Jr: The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 93: 440-447, 1961.

12. Tor M, Öztürk M, Altın R, Çımrın AH. Working conditions and pneumoconiosis in Turkish coal miners between 1985 and 2004: a report from Zonguldak coal basin, Turkey. Tüberküloz ve Toraks Dergisi 2010; 58(3): 252-260.

13. Castranova V, Vallyathan V: Silicosis and coal workers' pneumoconiosis; Environ Health Perspect 108 (Suppl 4): 675-684, 2000

14. Bowden DH: Macrophages, dust, and pulmonary diseases. Exp Lung Res 12(2): 89-107, 1987

15. Vallyathan V, Mega JF, Shi X, Dalal NS: Enhanced generation of free radicals from phagocytes induced by mineral dusts. Am J Respir Cell Mol Biol 6(4): 404-413, 1992

16. Srivastava PK, Singh Y, Misra UK: Pulmonary and hepatic glutathione levels, glutathione shuttle enzymes and lipid peroxidation in rats exposed intratracheally to coal fly ash. Biochem Int 17(3): 509-516, 1988

17. Perrin-Nadif R, Auburtin G, Dusch M, Porcher JM, Mur JM: Blood antioxidant enzymes as markers of exposure or effect in coal miners. Occup Environ Med 53(1): 41-45, 1996

18. Nadif R, Bourgkard E, Dusch M, Bernadac P, Bertrand JP, Mur JM, Pham QT: Relations between occupational exposure to coal mine dusts, erythrocyte catalase and Cu^{++}/Zn^{++} superoxide dismutase activities, and the severity of coal workers' pneumoconiosis. Occup Environ Med 55(8): 533-540, 1998

19. Arslan SO, Zerin M, Vural H and Coskun A: The effect of melatonin on bleomycin-induced pulmonary fibrosis in rats. J Pineal Res 32: 21-25, 2002.

20. Gosset P, Lassalle P, Vanhee D, Wallaert B, Aerts C, Voisin C, Tonnel AB: Production of tumor necrosis factor-alpha and interleukin-6 by human alveolar macrophages exposed in vitro to coal mine dust. Am J Respir Cell Mol Biol 5(5): 431-436, 1991

21. Vanhee D, Gosset P, Marquette CH, Wallaert B, Lafitte JJ, Gosselin B, Voisin C, Tonnel AB: Secretion and mRNA expression of TNF alpha and IL-6 in the lungs of pneumoconiosis patients. Am J Respir Crit Care Med 152(1): 298-306, 1995

22. Zhai R, Liu, G, Ge X, Bao W, Wu C, Yang, C, Liang D: Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), and their soluble receptors in coal workers' pneumoconiosis. Respir Med 96(10): 829-834, 2002

23. Yang LT, Liu X, Wu GH, Chen LF. Association between tumor necrosis factor-α -308 Gauss/A polymorphism and risk of silicosis and coal workers pneumoconiosis in Chinese population. Inhal Toxicol. 2018 May;30(6):213-217.

24. Arslan SO, Gelir E, Sayan H, Ozacmak VH. L-Arginine and melatonin interaction in rat intestinal ischemia--reperfusion. Fundam Clin Pharmacol. 2005 Oct;19(5):533-5.