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Evaluation of Protective Effect of Valethamate Bromide in Diesel Exhaust Induced Lung Damage in Rats

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Harshada Jadhav *, V. J. Chaware, R. P. Bhoite, V. K. Redasani

YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara, India.

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ABSTRACT

Epidemiological and experimental studies have suggested that diesel exhaust particles (DEP) may be involved in recent increases in lung diseases. In the present study, We investigated the effects of valethamte bromide in diesel exhaust-induced lung damage in rats. Valethamate bromide is an ester with a quaternary N atom, which by virtue of its anticholinergic, parasympatholytic and musculotropic action. The animals were randomized into 4 experimental groups. All animals in three groups except control group were exposed to diesel exhaust (4 hr/day, 5 days/week for 4 weeks). The animals were treated with valethamate bromide (16 mg/kg, p.o), N- acetylcysteine (200 mg/kg, p.o), simultaneously to the animals receiving DE treatment. After 28 days of study, the blood samples were taken directly by retro-orbital puncture. Six animals from each group were sacrificed in CO_2 chamber and lungs were observed. After DEP exposure the total number of cells, neutrophils, lymphocytes and macrophages levels in the BALF were increased in negative group as compare to control group. Treatment with valethamate bromide decreased the total number of cells, neutrophils, lymphocytes and macrophages in the BALF compared with negative group. The C-reactive protein level and haematological parameters were significantly greater in the negative group as compared to normal control group. Treatment with valethamate bromide decreased the level of C-reactive protein and hematological parameters as compared to negative group. Histopathological changes showed that infiltration of large numbers of leucocytes, emphysema, congestion and edema in the negative group as compare to control group and treatment with valethamate bromide showed that infiltration of small numbers of leucocytes, emphysema, congestion and edema as compare to negative group. Valethamate bromide makes it a promising drug for treating clinical and pathological abnormalities against Diesel exhaust induced lung damage in rats.

1. INTRODUCTION

It is well-recognized that air pollution is causally linked to a variety of clinical disorders afflicting the lungs [1]. Epidemiological studies have shown a positive relationship between exposure to ambient particulate matter (PM) and adverse respiratory health effects in humans [2,3] . Exposure to air pollutants generated by petrol- and diesel-burning engines aggravates the symptoms of respiratory diseases such as asthma and rhinitis [4,5]. Air pollutants expelled by diesel engine-powered automobiles include diesel exhaust particles (DEP), which are known to be major constituents of atmospheric PM in metropolitan areas. DEP has been linked to lung cancer, bronchitis [6], oedematous lung changes [7], and airway inflammation [8]. DEP enhances the manifestation of allergic asthma in a variety of murine models [9-12]. Moreover, short-term exposure to DEP reportedly induces an acute inflammatory response in humans as well as in animals. [1,7]

Chronic obstructive pulmonary disease (COPD) is a major healthcare problem, respiratory disease which is characterized by chronic airway inflammation, followed by reduction in lung function over time, and progressively impairment in quality of life, which is not fully reversible. COPD has high occurrence rates worldwide and is mainly caused not only by the inhalation of noxious substances, predominantly cigarette smoking but also by air pollution, especially in developing countries.[13]

Valethamate bromide or epidosin is form the group of 'Eosin' described by Steinmann (1954) [14], It is an ester with quaternary N atom, which by virtue of its anticholinergic, Parasympatholytic and Musculotropic action. However, no previous study has evaluated the efficacy of valethamate bromide in the treatment of lung diseases. In this study, we investigated the effect of an oral dose of valethamate bromide in diesel exhaust induced lung damage in rats. [15]

Fig. 1. Chemical structure of valethamate bromide

2. Materials and Methods

2.1 Chemicals and reagents

Valethamate Bromide was purchased from Acme Life Tech- LLP and N-Acetylcysteine (NAC) was purchased from Care Formulation Labs Pvt. Ltd.

2.2 Animals

Adult wistar rats (150-200 g) were used in the present study. These animals were procured from a registered breeder and acquainted in the quarantine area for one week. The animals were housed in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions of 22 +2°C temperature, 50+ 15% of relative humidity, (12 hr dark 12 hr light) cycle with free access to a pellet diet and water provided *adlibitum.*

2.3 DE Exposure

Fig. 2. Experimental equipment for diesel exhaust chamber.

i. Principle:

Diesel is burned in the primary chamber. It produces the smoke and that is directly exposed to the experimental animal in the secondary chamber.

ii. Construction:

The above apparatus consist of two cylindrical chamber of variable size. One is primary or combustion chamber (Diameter-15 cm and Height- 25 cm) and second is the lodging chamber (Diameter- 13 cm and Height-25 cm). Both the chambers are separated by 2-3 sieves. The combustion chamber is attached with sufficient air supply from aerator.

iii. Working:

Initially, a cotton plug of 4-6 gm is taken and dipped in 5 ml of diesel that is put in a beaker. This beaker is placed in a combustion chamber with the required air supply from the aerator through the pipe. Now the diesel is burned and the smoke is produced which passes through the sieve to the lodging chamber in which the animal is placed. 2-3 Sieves are placed to protect the animal from direct heat or flame.

2.4 Study protocol

The animals were exposed to diesel exhaust for 4 hr/day, 5 days/week for 4 weeks randomized into 4 experimental groups. All animals in the three groups except control group were exposed to diesel exhaust for 28 days. Group III and IV animals were treated with N-acetyl cysteine (200mg/kg, p.o), valethamate bromide (16 mg/kg, p.o), respectively to the animals receiving DE treatment. After 28 days of study, the blood samples were taken directly by retro-orbital puncture. Six animals from each group were sacrificed in a CO_2 chamber and lungs were observed, various biochemical parameters were evaluated.[16]

2.5. Bronchoalveolar lavage fluid (BALF) collection and analysis

The trachea was cannulated after exsanguination. The lungs were flushed with 1.2 ml sterile saline at 37°C instilled bilaterally using a syringe. The lavage fluid was harvested by gentle aspiration. This procedure was peated three times. For all treatment groups, an average of 90% of the instilled 3.6 ml was retrieved. The fluids from three lavages were combined, cooled to 4°C, and centrifuged at 300 × g for 10 min. Total cell counts were determined for fresh fluid specimens using a hemocytometer. Cytocentrifuge smears of lavaged cells were stained with Diff-Quick. Differential cell counts were determined by counting 300 cells under oil immersion using an Olympus AX80 light microscope. [17]

2.6 Blood cells count

At the end of the experiment period, with a EDTA-coated syringe, five ml blood sample was taken by retro-orbital puncture. Immediately after anaesthesia and exposing the animals chest. The leukocyte count was determined on blood samples diluted 1:10 in Turk solution by means of a Neubauer's hemacytometer. The Turk solution consisted of 1 mL of glacial acetic acid, 1 mL of Gentian Violet Solution 1% and 100 mL distilled water. To avoid aggregation of white blood cells, fresh blood always maintained on ice. Instantly, selected

smears were fixed with methanol and stained with Giemsa's solution, and then used for a differential count of the white blood cells. According to staining and morphological criteria, differential cell analysis was done under the light microscope by counting 100 cells, and the percentage of each cell type was calculated. The erythrocyte number (RBC) was counted in Neubauer's hemocytometer after the sample was diluted (1:200) in a saline solution. For determining the platelet count, whole blood was diluted with 1% ammonium oxalate solution. The standard dilution for platelet counts was 1:100. The dilution was mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution was mounted on a hemacytometer. The cells were allowed to settle and then were counted in a specific area of the hemacytometer chamber under the light microscope. [18]

2.7 C reactive protein

C-reactive protein (CRP), a member of the pentraxin family of plasma proteins, is one of the most distinctive acute phase reactants. In response to inflammation, cell damage or tissue injury, plasma level of CRP rapidly and dramatically increases up to 1000-fold, a phenomenon that has been used for years to monitor infections and many destructive/inflammatory conditions. The magnitude of CRP increase usually correlates with the severity of injury or inflammation and reflects an important physiological role of this interesting but still under-investigated protein. It is now generally accepted that CRP is involved in host defense and inflammation. However, the exact function of this protein in health and disease remains unclear. Many studies have demonstrated that in different pathophysiological conditions, CRP might be involved in the regulation of lung function and may participate in the pathogenesis of various pulmonary disorders. The fluctuation of CRP concentrations in both alveolar fluid and serum associated with different pulmonary diseases suggests its important role in lung biology.

C-reactive protein (CRP) is a substance the liver produces in response to inflammation.

A high level of CRP in the blood can be a marker of inflammation. A wide variety of conditions can cause it, from an infection to cancer. However, the CRP test is an extremely nonspecific test. CRP levels can be elevated in many inflammatory conditions. While the regular C-reactive test can help uncover different diseases that cause inflammation by measuring high levels of protein, the hs-CRP test measures lower (but still elevated) levels of protein, which can signal the risk of heart disease and stroke.[19]

This test is done via a blood sample. Rat CRP ELISA kit:

Test Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat CRP. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat CRP and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat CRP, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in colour. The enzyme-substrate reaction is terminated by the addition of a stop solution and the colour turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat CRP. You can calculate the concentration of Rat CRP in the samples by comparing the OD of the samples to the standard curve.[20]

2.8 Histopathological examination

It is the science or art of preparing organs, tissues or tissue components for microscopic observations and study. This technique includes the study of the tissues collected from living animal at operation (biopsy) or from recently dead animal (necropsy) during post mortem examination.

After exsanguination, the lungs were fixed by intratracheal instillation with 10 % neutral phosphate-buffered formalin (pH 7.4) and embedded in paraffin. Sections of 4 µm thickness were routinely processed with hematoxylin and eosin stain.[21]

2.9 Statistical analysis

Data are expressed as mean ± standard deviation (SD) values. The data were analyzed for significance using one-way ANOVA followed by Newman–Keuls test to adjust for multiple comparisons.[22]

3. Results

3.1 Bronchoalveolar lavage fluid (BALF) collection and analysis.

We first examined whether valethamate bromide could inhibit lung damage induced by DEP exposure. The total number of cells, neutrophils, lymphocytes and macrophages in the BALF was significantly greater in the negative group (DEP 4hr/day, 5 days/week for 4 weeks) as compared with control group. Treatment with valethamate bromide decreased the total number of cells, neutrophils, lymphocytes and macrophages in the BALF as compared with negative group.

Table. 1. Effect of valethamate bromide on the cellular profile of the bronchoalveolar lavage fluid in rat instilled with diesel exhaust.

Parameters	**Control Group**	**Negative Group (DEP)**	**Standard Group (N-acetylcysteine)**	**Test Group (Valethamate bromide)**
TLC – No of cells ($\times 10^4$ in total BAL fluid)	1.3 ± 0.23	2.3 ± 0.41	1.6 ± 0.28	1.5 ± 0.27
Neutrophils ($\times 10^4$ in total BAL fluid)	0.04 ± 0.076	0.12 ± 0.022	0.05 ± 0.009	0.06 ± 0.011
Lymphocytes ($\times 10^2$)	1.2 ± 0.22	1.9 ± 0.36	1.3 ± 0.24	1.4 ± 0.26
Macrophages ($\times 10^4$)	1.4 ± 0.25	2.2 ± 0.39	1.3 ± 0.23	1.2 ± 0.21

Values are expressed as the mean ± SD a-$p < 0.05$ significant difference compared to DEP. Control, animals received normal saline solution. DEP: animals received diesel exhaust particles (4 hr/ day, 5 days/week for 4 weeks), Standard: diesel exhaust induced, 4hr/day and treated with NAC (200 mg/kg), Test: Diesel exhaust induced, (4hr/day, 5 days/week for 4 weeks) and treated with Valethamate Bromide (16 mg/kg).

3.2 Haematological Analysis

In this study the number of erythrocytes and hemoglobin levels was calculated and decreased in the number of RBCs and haemoglobin molecules was observed in negative group (DE 4hr/day, 5 days/week for 4 weeks) when compared with control group. Moreover a significant increased in the number of RBCs and haemoglobin levels was observed in the test group animals when compared with negative group. The number of white blood cells, platelets, neutrophils, lymphocytes, eosinophils was increased in negative group as compared with control group. When animals treated with valethamate bromide, the number of white blood cells, platelets, neutrophils, lymphocytes, eosinophils were found to be decreased as compared with negative group.

Table. 2. Effect of valethamate bromide on haematological parameters in rat instilled with diesel exhaust.

Parameters	**Control Group**	**Negative Group (DEP)**	**Standard Group (N-acetylcysteine)**	**Test Group (Valethamate bromide)**
Haemoglobin (gm%)	13.6 ± 2.17	9.2 ± 1.65	12.2 ± 2.19	12.4 ± 2.23
RBC count (million/cumm)	5.72 ± 1.28	3.17 ± 0.634	4.50 ± 1.2	4.30 ± 0.817
White blood cells (cells /cumm)	7789 ± 1407.02	12000 ± 2160	8442 ± 1519.56	8552 ± 1539.36
Neutrophils (%)	42.11 ± 7.57	50.12 ± 9.02	43.10 ± 7.75	42.24 ± 7.60
Lymphocytes (%)	38.47 ± 6.92	64.25 ± 11.56	39.12 ± 7.04	42.24 ± 7.60
Eosinophils (%)	3.7 ± 0.70	6.4 ± 1.21	3.6 ± 0.68	3.2 ± 0.60
Platelets (cells /cumm)	42000 ± 7980	75000 ± 14250	15250 ± 2897	11500 ± 2185

Values are expressed as the mean ± SD a-p < 0.05 significant difference compared to DEP. Control, animals received normal saline solution. DEP: animals received diesel exhaust particles (4hr/ day, 5 days/week for 4 weeks), Standard: diesel exhaust induced, (4hr/day,5

days/week for 4 weeks) and treated with NAC (200 mg/kg), Test: Diesel exhaust induced (4hr/day,5 days/week for 4 weeks), and treated with Valethamate Bromide (16 mg/kg).

3.3 Effect of valethamate bromide on serum C Reactive protein (CRP) against diesel exhaust-induced lung damage in rats.

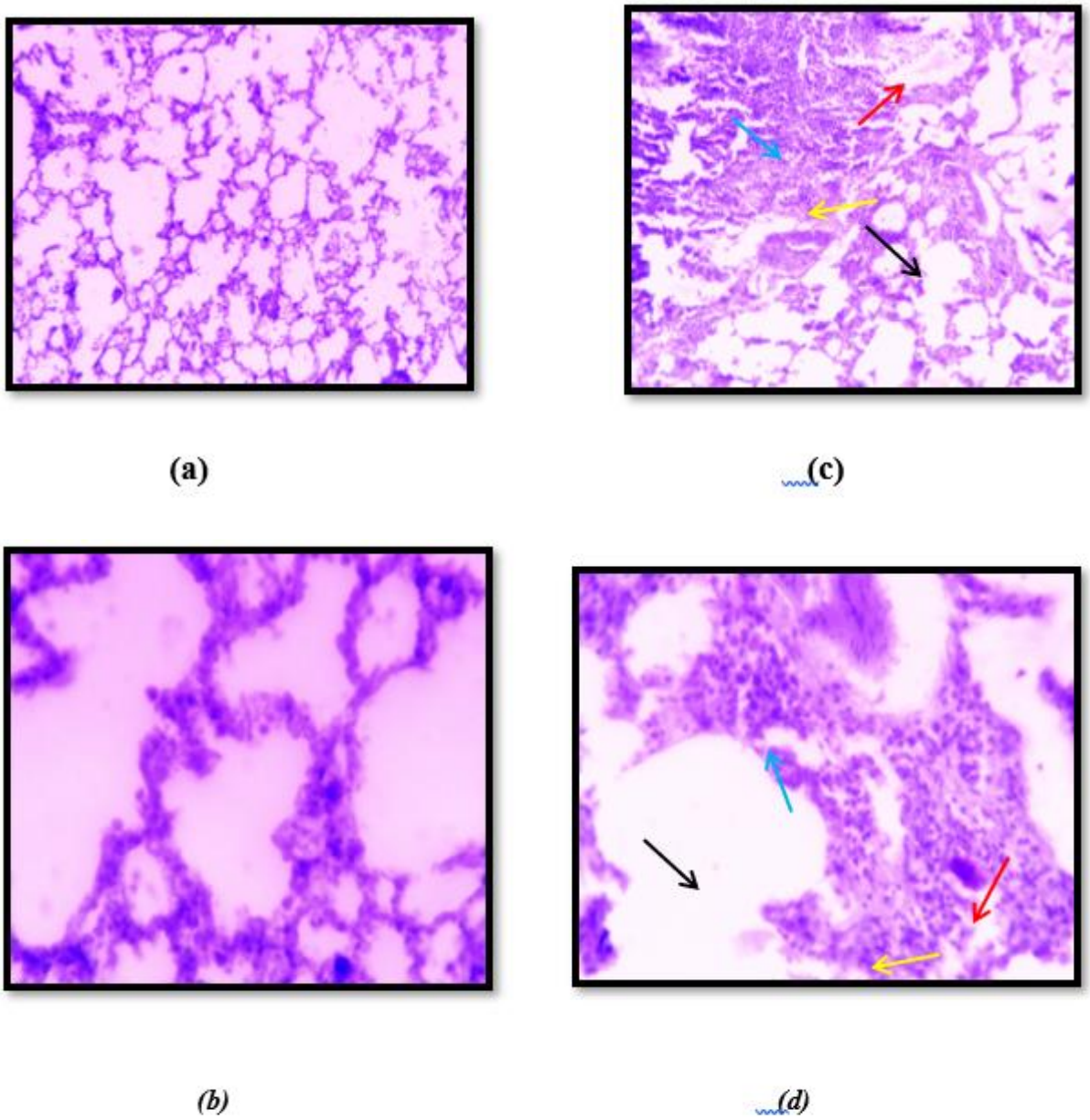
The negative group (diesel exhaust, 4hr/day, 5 days/week for 4 weeks) compared with control group only showed significant increase in CRP level. Animals treated with standard drug (N- acetyl cysteine 200mg/kg, p.o) showed significant decrease in CRP level as compared with the negative group. Animals treated with test drug (Valethamate bromide 16mg/kg, p.o) showed significant decrease in CRP level as compared with negative group.

Table.3. Effect of valethamate bromide on serum C Reactive protein (CRP) against diesel exhaust-induced lung damage in rats.

Parameters	**Control Group**	**Negative Group (DEP)**	**Standard Group (N-acetylcysteine)**	**Test Group (Valethamate bromide)**
C reactive Protein (mg/ml)	4.2 ± 0.79	8.2 ± 1.55	4.6 ± 0.87	4.3 ± 0.81

Values are expressed as the mean ± SD $p < 0.05$ significant difference compared to DEP. Control, animals received normal saline solution. DEP: animals received diesel exhaust particles (4hr/ day, 5 days /week for 4 weeks), Standard: diesel exhaust induced, 4hr/day and treated with NAC (200 mg/kg), Test: Diesel exhaust induced, and treated with Valethamate Bromide (16 mg/kg).

3.4 Histopathology of lungs.



(a) (c)

(b) (d)

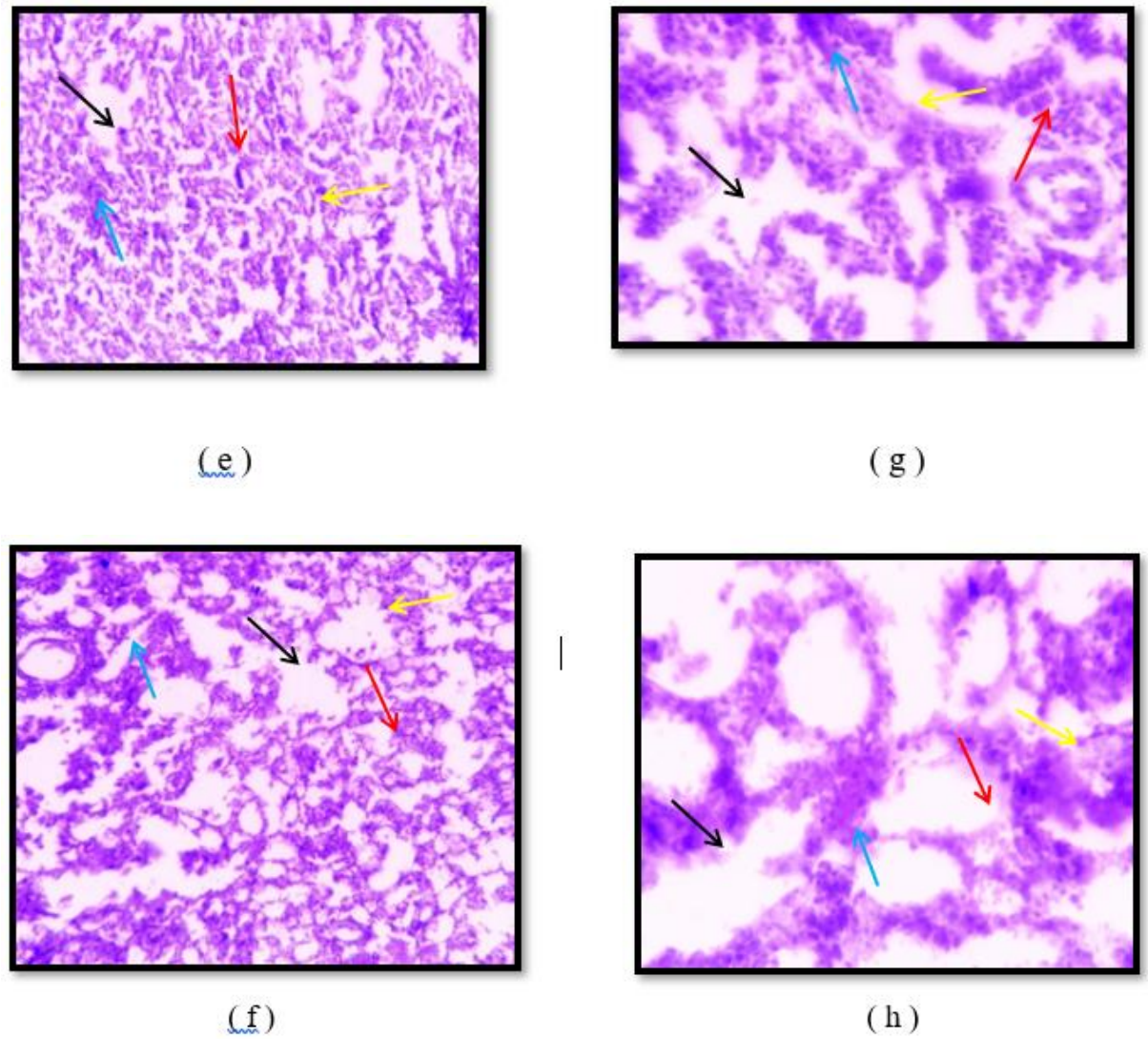


Fig. 3. Valethamate bromide inhibits histological changes in the lung induced by diesel exhaust particles (DEP). We used Wistar rats randomized into four experimental groups. Group IV was treated with valethamate bromide for 28 days at a dose of 16 mg/kg, p.o. lung specimens were stained with hematological and eosin. Lung section was obtained from rats instilled with (a and b) vehicle alone, (c and d) DEP, (e and f) treatment with N-acetylcysteine, (g and h) treatment with valethamate bromide. Original magnification (a) × 10 , (b) × 40 , (c, e and g) × 100 , (d, f and h) × 400.

Valethamate bromide inhibits lung damage induced by DEP

Photograph showing infiltration of large numbers of leucocytes (blue arrow), emphysema (black arrow), congestion and (red arrow) edema (yellow arrow) H&E stain,

(Pathological grade: ++++) in the negative group as compared with control group.

Treatment with valethamate bromide showed infiltration of small numbers of leucocytes (blue arrow), emphysema (black arrow), congestion and (red arrow) edema (yellow arrow) H&E stain as compared with negative group.

(Pathological grade: ++)

4. Discussion

Chronic obstructive pulmonary disease (COPD) is a major health care problem, respiratory disease which is characterized by chronic airway inflammation, followed by reduction in lung function over time, and progressively impairment in quality of life, which is not fully reversible. COPD has high occurrence rates worldwide and is mainly caused not only by the inhalation of noxious substances, predominantly cigarette smoking, but also by air pollution, precisely in developing countries. It is mainly caused due to cigarette smoking, also by air pollution. It is currently the fifth leading cause of death worldwide, but according to the WHO project it will become third leading cause by 2030.[23] We observed that the valethamate bromide inhibited the emphysema, congestion, oedema and infiltration of large numbers of leucocytes. We investigated the effect of valethamate bromide on DEP- induced lungs injury. DEP induced lungs injury was inhibited by daily oral supplementation with valethamate bromide. The beneficial effect of valethamate bromide were associated with the improvement in the level of hemogram, C-reactive protein, bronchoalveolar lavage fluid.

DEP act as nonspecific airway irritants at relatively high levels. These particles lead to cause damage to lungs tissue and initiate the immunological responses. Gradually leads to release of specific cytokines, chemokines, immunoglobulins, leukotrienes/ prostaglandins and adhesion molecules. Release of these mediators of the allergic and inflammatory response initiate a cascade that can culminate in airway inflammation. BAL fluid exhibits the cellular and biochemical alterations of inflammation and lung injury in response to various toxic agents. BAL fluid composed of total leucocyte cells, neutrophils, lymphocytes, macrophages etc. In the present study, the total number of cells in BAL fluid were significantly greater in the negative group (diesel exhaust induced, 4hr/day,5 days/week for 4 weeks) as compared with control group. Animals treated with test drug (Valethamate bromide 16 mg/kg) showed significant decrease in the total number of cells in BAL fluid as compared with negative group.[24] BAL test is also helpful for the detection of tuberculosis, bacterial pneumonia, fungal infection etc.

DE particles are capable of entering into the lung cells and blood cells similarly as it happens in case of macrophages. Particulate matter can translocate through the respiratory epithelium towards circulation and subsequently exert toxicity to vascular endothelium, interact with circulatory cells such as erythrocytes, and platelets and cause alteration of blood composition. DE particles cause to form adhesion molecules including inter-cellular adhesion molecule or the direct toxicity of particles on circulating erythrocytes. DE is a major contributor to traffic related pollution and contain higher fine particulate content. Therefore the present study provide some support to the hypothesis that acute exposure to traffic related air pollution initiates haemoconcentration, systemic inflammatory process activating the endothelial blood cell interface and increasing circulating platelets.[25] In this study the number of erythrocytes and haemoglobin molecules were calculated and decrease in the number of RBCs and haemoglobin molecules was observed in negative group (DE 4hr/day,5 days/week for 4 weeks) when compared control group. Moreover, a significant increase in the number of RBCs and haemoglobin molecules was observed in the test group animals when compared with negative group.

C-reactive protein (CRP), a marker of systemic inflammation, has been used in chronic inflammatory diseases. Circulating levels of CRP are known to increase in response to infection and tissue damage. Higher levels of serum CRP were associated with impaired lung function. In present study we found that the serum CRP levels were increased in negative group (DEP 4hr/day,5 days/week for 4 weeks) as compared to control group. Moreover, a significant decreased in the level of CRP were observed in the test group animals when compared with negative group. CRP is a pentameric protein synthesized by the liver, whose level rises in response to inflammation. CRP is an acute- phase reactant protein that is primarily induced by the II-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process. It has been demonstrated to have some protective properties in animal studies on lung tissue in alveolitis by reducing neutrophil-mediated damage to the alveoli and protein leakage into the lung. CRP has both proinflammatory and anti-inflammatory properties. It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin. It can activate the classic complement pathway and also activate phagocytic cells via Fc receptors to expedite the removal of cellular debris and damaged cells and foreign pathogens. The fluctuation of

CRP concentrations in both alveolar fluid and serum associated with COPD suggest it's important role in lung physiology. [26,27]

Histopathology is the microscopic examination of biological tissues to observe the appearance of diseased cells and tissues in very fine detail. In the present study, histopathological examinations of lung tissues were done.

In the histopathological study of the lung tissues proved DEPS caused the infiltration of leucocytes (blue arrow), emphysema (black arrow), congestion (red arrow) and edema (yellow arrow), Treatment with valethamate bromide showed significant reduction in the infiltration of leucocytes (blue arrow), emphysema (black arrow), congestion (red arrow) and edema (yellow arrow).

5. Conclusion

Diesel exhaust successfully induced lung damage in rats via self designed DE apparatus. DEPs elevated C- -reactive protein, bronchoalveolar lavage fluid, caused structural changes or damage to pulmonary tissues and cells (alveoli).

Valethamate bromide therapy with dose 16 mg/kg, p.o was observed to be beneficial similar to standard drug N-acetylcysteine 200 mg /kg, p.o in protecting and normalizing affected lung tissues in diesel exhaust-induced lung damage in rats.

Valethamate bromide successfully restored the blood profile and BAL by normalizing the levels of RBCs, hemoglobin, platelets, total leucocytic count, neutrophils, lymphocytes, monocytes. macrophages. Valethamate bromide also prevented the structural changes and damage (alveolar enlargement) produced in pulmonary tissues by DEPs. Valethamate bromide reduced the C-reactive protein level.

Valethamate bromide makes it a promising drug for treating clinical and pathological abnormalities against diesel exhaust-induced lung damage in rats.

REFERENCES

[1] Salvi, S.; Blomberg, A.; Rudell, B.; Kelly, F.; Sandstrom, T.; Holgate, S. T.; Frew, A. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. Am. J. Respir. Crit. Care Med. 159:702–709; 1999.
[2] Dockery, D. W.; Pope, C. A. III; Xu, X.; Spengler, J. D.; Ware, J. H.; Fay, M. E.; Ferris, B. G. Jr; Speizer, F. E. An association between air pollution and mortality in six U.S. cities. N. Engl. J. Med. 329:1753–1759; 1993.

[3] Sarnat, J. A.; Schwartz, J.; Catalano, P. J.; Suh, H. H. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? Environ. Health Perspect. 109:1053–1061; 2001.
[4] Nicolai, T. Environmental air pollution and lung disease in children. Monaldi. Arch. Chest. Dis. 54:475–478; 1999.
[5] Segala, C.; Fauroux, B.; Just, J.; Pascual, L.; Grimfeld, A.; Neukirch, F. Short-term effect of winter air pollution on respiratory health of asthmatic children in Paris. Eur. Respir. J. 11:677– 685; 1998.
[6] McClellan, R. O. Health effects of exposure to diesel exhaust particles. Annu. Rev. Pharmacology Toxicol. 27:279 –300; 1987.
[7] Ichinose, T.; Furuyama, A.; Sagai, M. Biological effects of diesel exhaust particles (DEP). II. Acute toxicity of DEP introduced into lung by intratracheal instillation. Toxicology 99:153–167; 1995.
[8] Sagai, M.; Furuyama, A.; Ichinose, T. Biological effects of diesel exhaust particles (DEP). III. Pathogenesis of asthma-like symptoms in mice. Free Radic. Biol. Med. 21:199 –209; 1996.
[9] Takano, H.; Yoshikawa, T.; Ichinose, T.; Miyabara, Y.; Imaoka, K.; Sagai, M. Diesel exhaust particles enhance antigen-induced airway inflammation and local cytokine expression in mice. Am. J. Respir. Crit. Care Med. 156:36 – 42; 1997.
[10] Miyabara, Y.; Takano, H.; Ichinose, T.; Lim, H. B.; Sagai, M. Diesel exhaust enhances allergic airway inflammation and hyperresponsiveness in mice. Am. J. Respir. Crit. Care Med. 157: 1138 –1144; 1998.
[11] Takano, H.; Ichinose, T.; Miyabara, Y.; Yoshikawa, T.; Sagai, M. Diesel exhaust particles enhance airway responsiveness following allergen exposure in mice. Immunopharmacological Immunotoxicology. 20:329 – 336; 1998.
[12] Takano, H.; Lim, H. B.; Miyabara, Y.; Ichinose, T.; Yoshikawa, T.; Sagai, M. Manipulation of the L-arginine-nitric oxide pathway in airway inflammation induced by diesel exhaust particles in mice. Toxicology 139:19 –26; 1999.
[13] Series, I. Chronic Obstructive Pulmonary Disease (COPD), 199, 1-2 (2019).
[14] Baser A, Desal SV, Acceleration of labor by epidosin. J Obstet Gynaecol India 1993; 43 (2): 217-220.
[15] Leroy MI, Luginer G, Isolation and characterization of rolipram sensitive cAMP – Specific phosphodiesterase [type IV PDE] in human term myometrium. Cell Signals 1994; 6: 405-412.
[16] Reddy R. Gottipolu,1,* J. Grace Wallenborn,2 Edward D. Karoly,3 Mette C. Schladweiler,1 Allen D. Ledbetter,1 Todd Krantz,1 William P. Linak,4 Abraham Nyska,5 One-Month Diesel Exhaust Inhalation Produces Hypertensive Gene Expression Pattern in Healthy Rats. Environ Health Perspect 117:38–46 (2009). doi:10.1289/ehp.11647 available via http://dx.doi.org/ [Online 12 September 2008]
[17] Chiaki Sanbongi dt al. 2003: Rosmarinic acid inhibits lung injury induced by diesel exhaust particles ,2003.
[18] Somayyeh Vosooghi, Maryam Mahmoudabady, Ali Neamati*, Heydar Aghababa Avicenna Preventive effects of hydroalcoholic extract of saffron on haematological parameters of experimental asthmatic rats. Journal of Phytomedicine Received: Feb 6, 2013; Accepted: Mar 28, 2013 Vol. 3, No. 3, Summer 2013, 279-287.
[19] Panel Marianna Agassandian [a], Galina V. Shurin [a], Yang Ma [a], Michael R. Shurin [a b] C-reactive protein and lung diseases https://doi.org/10.1016/j.biocel.2014.05.016
[20] Elabscience CRP (C- Reactive Protein) ELISA Kit , Test principle.
[21] Chiaki Sanbongi dt al. 2003: Rosmarinic acid inhibits lung injury induced by diesel exhaust particles ,2003.
[22] Ying-Ji Li,1,* Takako Shimizu,1 Yusuke Shinkai,2 et.al. Nrf2 Lowers the Risk of Lung Injury via Modulating the Airway Innate Immune Response Induced by Diesel Exhaust in Mice.(Oct- 2020).
[23] Devine, J. F. Chronic obstructive pulmonary disease: an overview. Am. Heal. drug benefits 1, 34-42 (2008).
[24] Chiaki Sanbongi dt al. 2003 : Rosmarinic acid inhibits lung injury induced by diesel exhaust particles ,2003.
[25] Rajini M Krishnan, Jeffery H Sullivan, Chris Carlsten A randomized cross over study of inhalation of diesel exhaust, hematological indices and endothelial markers, (2013)
[26] Panel Marianna Agassandian [a], Galina V. Shurin [a], Yang Ma [a], Michael R. Shurin [a b] C reactive protein and lung diseases. https://doi.org/10.1016/j.biocel.2014.05.016

[27] Liping Zhang et, al C- reactive protein gene polymorphisms correlated with serum CRP levels of diesel engine exhaust -Exposed workers.(1998)