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Evaluating a herbal formulation for particulate matter induced lung tissue degeneration in wistar rat

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Abstract:

Particulate Matter (PM) is the biggest challenge for human health as the lungs shows its deleterious impacts even in early pathogenesis. It is imperative to treat the lung tissues with effective and with minimum side effects of the medicine. This study is carried out to evaluate a herbal formulation which included eight plant samples '*Angelica archangelica (leaf), Dioscorea bulbifera (Root), Fagopyrum dibotrys (Root), Myrtus communis Linn (Leaf), Nasturtum offcinalea* (whole Plant), *Perilla frutescens* (Leaf and Seed), *Catharanthus roseus* (Whole Plant) and *Solanum xanthocarpum* (Root). The therapeutic actions were observed against particulate matter concentrations (artificially generated PM exposure) induced lungs tissue degeneration at various dose 100, 200 and 400 mg/kilogram in wistar albino rats for 30 days. Blood sample collected terminally on 30th day has been gone through the hematology test. All surviving animals have been euthanized by high dose of anaesthetics and subjected to a lung necropsy examination at the end of the treatment period Differential cell count in blood have been analysed. As well as in Bronchoalveolar lavage fluid (BALF) also. That has been reduced progressively in all test groups (<0.05, <0.01, <0.001). Histopathology of the lungs reflected inflammatory changes, deform alveolar space, edema and condense of the intra-bronchial wall. In the induced control group and in the treatment group @ doses of 100, 200, and 400 mg/kilogram progressive reduced inflammation alveolar space, and edema. The results showed that the formulation was found therapeutic active against degenerated lung tissue induced by particulate matter in wistar albino rats most effective at a dose of 400 mg/kilogram during 30 days.

Keywords: Particulate matter, herb, lung tissue, inflammation

Introduction:

Air pollution is deleterious for human health because it is a mixture of gaseous components and (PM) particulate matter constituents. Based on the source, the main components presents in PM are carbonaceous material, reactive metal, nitrates, polycyclic hydrocarbons (aromatic), sulphates, endotoxin, and some metals such as iron, nickel, zinc, vanadium and copper. Main source of PM is fossil fuel combustion . PM is classified on the basis of particle size such as PM_{10} (<10µm), $PM_{2.5}$ (<2.5µm). PM_{10} having diameter of less than 10µm. Fine particles $PM_{2.5}$ having diameter of less than 2.5µm. Ultrafine particulate matter called $PM_{0.1}$ (<0.1µm) (Hamanaka and Mutlu, 2018). PM have the potent property of being oxidative and leave a toxic effect on human body respiratory system and blood circulatory system. Particulate matter is the source of oxidative stress when it gets inhaled by the susceptible member of population. This oxidative stress takes place within the lung cells. And it respond in early pathogenesis which is dangerous for lungs. It disturbs the quantity of inflammatory cells into the lungs and it starts to generate large amount of free radicals, this over run of radicals in absent of any antioxidant can cause lung injury due to ambush of free radicals on the component of lung tissues. (Kelly and F. J., 2003). After exposure of PM many pro-inflammatory cytokines produce oxidative stress in lung tissue and lung epithelial cell because PM is strong oxidant and it increase the circulation of IL-1β, IL-6, and TNF-α

markers in lung tissues (Chang et. al., 2019). Inflammation increases in neutrophils in BALF the at lower bronchial lavage, BAL (Saxon et. al., 2005). There are many alternates for treatment of respiratory and lung diseases. In allopathy medication system steroids such as udesonide and formoterol are mainly used to treat lung diseases but it leaves side effects after a long use (Foster et.al., 2012). In traditional medicine system many options such as Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy are available to treat lung diseases. These treatments rarely shows side effect and these are cost effective too. Concept of treatment with Ayurveda is based on holistic approach in, and it has given more consideration than allopathic medicines (Singh, 2016). In this study we conducted an efficacy study of novel herbal formulation against particulate matter induced lung tissue degeneration. This formulation included eight plant samples '*Angelica archangelica (leaf), Dioscorea bulbifera (Root), Fagopyrum dibotrys (Root), Myrtus communis Linn (Leaf), Nasturtum offcinalea* (whole Plant), *Perilla frutescens* (Leaf and Seed), *Catharanthus roseus* (Whole Plant) and *Solanum xanthocarpum* (Root). The efficacy of novel herbal formulation is evaluated in reference with CBC, BALF and histopathological study of lungs.

Methodology:

For this study 24 healthy albino wistar rats have been taken. These wistar albino rats were placed in acrylic chamber and air (Particulate matter) were incubated in the chamber with help of diesel generator and compassion of diesel generator Exhausted air was estimated with Air Quality Monitor BIAOLING Accurate Tester (carbon dioxide, formaldehyde, total volatile organic compounds, PM_{10} and $PM_{2.5}$. Lung Toxicity was developed after three-month exposure of diesel generator exhausted air for 3 hours per day. The animals have been divided into four groups, with six rats each.

Dose administration pattern:

Group 1- 6 wistar strain albino rats received distilled water 5ml/kg/oral for 30 days.

Group 2- 6 wistar strain albino rats received test formulation 100 mg/kg/oral for 30 days.

Group 3- 6 wistar strain albino rats received test formulation 200 mg/kg/oral for 30 days.

Group 4- 6 wistar strain albino rats received test formulation 400 mg/kg/oral for 30 days.

Blood sample collection and Isolation of Lungs:

Blood sample was collected from retro-orbital plexus of animal for CBC (complete blood count) analysis terminally on 30^{th} day. CBC was measured through hematology analyser of Genrui Biotech KT-6400.

After Euthanasia on 30^{th} day lungs have been isolated for histopathology and BALF analysis.

Histopathological analysis:

Isolated lungs put in buffet formalin for 24 hours. After that 12 hours washing have been done in running water. This washed lung tissue has been put in tissue processer. The tissue has been gone through 12 steps on 12 stations on tissue processer shown in table1.

Table 1: Tissue processing process

Process	Duration
Alcohol 70%	60 minutes
Alcohol 80%	60 minutes
Alcohol 90%	60 minutes
Absolute alcohol (first time)	60 minutes
Absolute alcohol (second time)	60 minutes

Absolute alcohol (third time)	60 minutes
Acetone I	15 minutes
Acetone II	15 minutes
Benzene I	15 minutes
Benzene II	15 minutes
Paraffin wax (60-62°C)	overnight
Paraffin wax (60-62°C	120 minutes

The processed tissues were embedded in "L" shaped module filled with paraffin wax and allowed to fix followed by casting. These tissue blocks were fixed to metal object holder. After that the staining was done shown in table 2.

Table 2: Staining process

Process	Duration
Xylene (first time)	15 minutes
Xylene (second time)	15 minutes
Xylene (third time time)	15 minutes
Absolute alcohol (first time)	2 minutes
Absolute alcohol (first time)	2 minutes
Absolute alcohol (first time)	2 minutes
90% Alcohol	2 minutes
70% Alcohol	2 minutes
Distilled Water	2 minutes
Haematoxylin	2 minutes
Running tap water	30 second
Dip in acid alcohol	5 second
Running water	30 second
Ammonia water	5 second
Wash in running water	30 second
Stain in Eosin (1%)	30 second
90 % Alcohol	Rinse
Absolute Alcohol (first time)	Rinse
Absolute Alcohol (second time)	2 minutes
Absolute Alcohol (third time)	2 minutes
Air dry slide	Till completely get dry
Xylene (first time)	5 minutes
Xylene (second time)	5 minutes
Xylene (third time)	5 minutes
Xylene (fourth time)	5 minutes

Finally slide mouth was mounted with DPX (DistreneDibutylPhthalate Xylene). Microscopic examination (Slaoui and Fiette, 2011).

Ethical Clearance:

The experimental protocol form B was submitted to the animal ethics committee of Bilwal Medchem And Reseaerch Laboratory Pvt. Ltd. H-9 SKS Reengus Industrial Area, Reengus, Rajasthan and approval was obtained for conducting the Experiment CPCSEA Registration NO 2005/PO/RcBt/S/18/CPCSEA. [Approval date: 31th January 2022,; Approval Number: BMRL/AD/CPCSEA/IAEC/2022/1/3, IAEC].

Result and Discussion:

Table 3: CBC evaluation

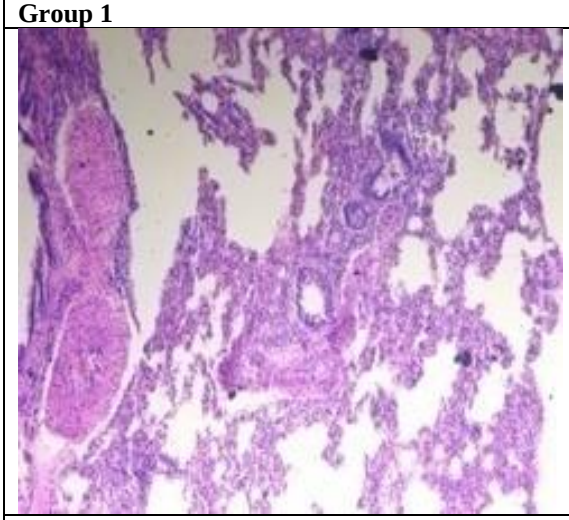
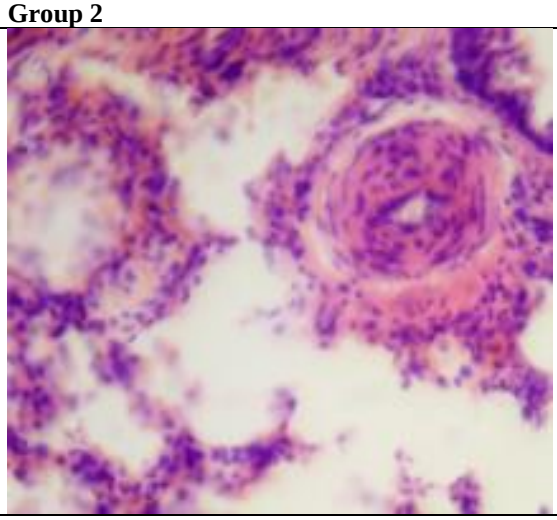
Parameters	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM	Group 4 Mean±SEM
WBC(10^9/L)	4.97±0.33	5.72±0.36	5.28±0.39	4.22±0.24
Neutrophils (%)	51.04±1.66	40.31±0.57***	35.96±1.22****	23.63±2.52****
Eosinophils (%)	6.78±0.19	5.95±0.28	4.77±0.34****	2.98±0.11****
Lymphocytes (%)	42.96±1.13	48.40±1.45	53.25±2.14**	64.93±2.28****
Monocytes (%)	3.85±0.28	3.38±0.24	2.93±0.18*	2.63±0.15**
Platelet Count (10^9/L)	323.33±29.71	388.00±25.18	405.00±28.52	369.50±20.86

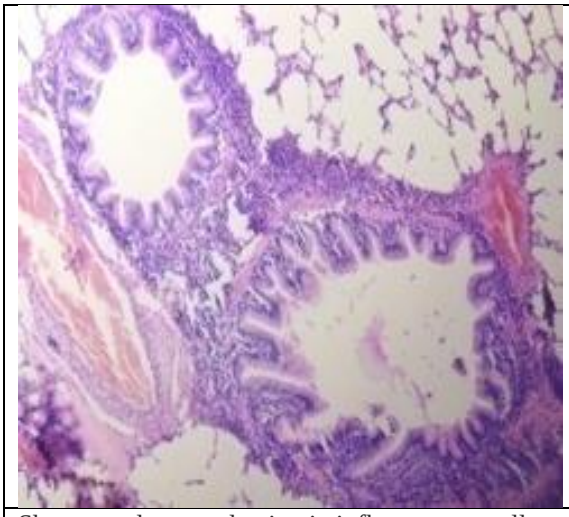
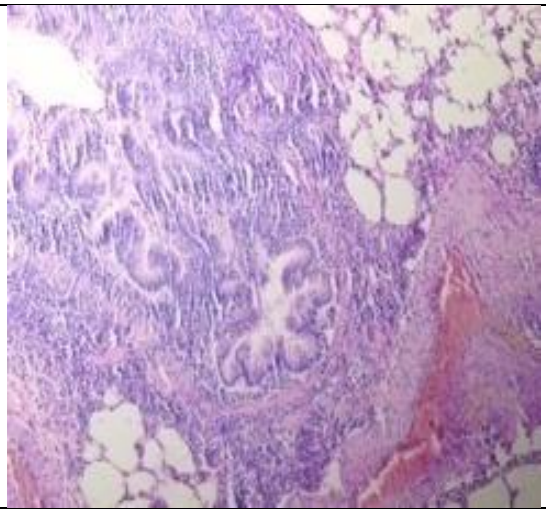
Table 3: Evaluation of leukocyte count in BALF

Parameters	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM	Group 4 Mean±SEM
Neutrophils (%)	43.42±0.94	38.54±1.14	30.28±2.38****	22.20±0.88****
Eosinophils (%)	9.24±0.53	7.35±0.29**	5.04±0.29****	3.48±0.25****
Lymphocytes (%)	45.45±1.56	54.01±1.36***	62.94±1.52****	70.84±1.10****
Monocytes (%)	3.94±0.31	3.33±0.24	2.41±0.12***	2.09±0.22****

*=p value <0.05, **=p value <0.01, ***= p value <0.001, ****= p value <0.0001

Table 4: lung tissue histopathology

Group 1	**Group 2**
	
Shows inflammatory changes, deformed alveolar space, edema and condensed intra-bronchial wall.	Shows mild reduction in inflammatory cell infiltration, thin intra-bronchial walls, and alveolar space
Group 3	**Group 4**

	
Shows moderate reduction in inflammatory cell infiltration, thin intra-bronchial walls, and alveolar space	Shows moderate to high reduction in inflammatory cell infiltration, thin intra-bronchial walls, and alveolar space

After 30th day of dosing all four groups were evaluated on basis of complete blood count, leukocyte count in BALF and histopathology study of lungs with reference of group 1(control group).

CBC analysis:

In CBC the composed WBC, Neutrophils, Eosinophils, Lymphocytes, Monocytes, Platelet Count were observed. It was revealed that there are no significant changes has been seen in WBC and platelet count with reference of group 1 because after pollution exposure there are no changes in WBC and platelet count so there is no probability of variation in parameter after dose administration. In Neutrophils, group 2, 3 and 4 are showing significant changes after treatment. Elevated number of neutrophils due to pollution exposure are changing in decreasing manner after treatment. Eosinophils and Lymphocytes are showing significant changes in group 3 and 4, these are showing changes in decreasing and increasing manner respectively. Monocytes are showing significant moderate changes in decreasing manner after treatment.

BALF analysis:

Another parameter, leukocyte count in BALF included Neutrophils, Eosinophils, Lymphocytes, Monocytes are showing significant changes (p value < 0.0001 in group 4).

Histopathological analysis:

After histopathology of lungs tissue group 1 showed inflammatory changes, deformed alveolar space, edema and condensed intra-bronchial wall. This inflammation caused due to the external factor of particulate matter that activated the inflammatory cells such as neutrophils, lymphocytes, and eosinophils. Activation of these cells may generate different inflammatory mediator such as tumour necrosis factor (TNF-α), interleukins, IL-1β, IL-4, IL-5, and IL-6, histamine, leukotrienes, and nitric oxide . These mediators are related to mucus hypersecretion, edema, lung injury, lung cell remodeling, loss of lung function and inflammation. Group 1 was untreated as it was control group while group 2, 3 and 4 showed reduction in inflammatory cell infiltration, comparatively thin intra-bronchial walls, and alveolar space after treatment. This formulation may effectively inhibited or suppressed the over production of pro-inflammatory cytokines such as IL-6 and IL-1β, and ROS.

Improvement in lung tissues are increased with increased dose level. In group 4 was observed with clear improvement in lung tissues after 30-day treatment.

Conclusion:

After the completion of scheduled dose period the evaluation parameter have been concluded as the efficiency indicator of herbal formulation in treatment of lung tissue degeneration and leukocytes count in BALF and CBC. Although doses have been administered on level of 100, 200, and 400 mg/kg for group 2, 3 and 4 respectively. All level of doses showed significant improvement in all parameters (CBC, BALF analysis, and histopathology of lungs) but on dose level 400 mg/kg in group 4 observed the most effective. Hence it is proved in this investigation that the novel herbal formulation found therapeutically active against degenerated lung tissue induced by particulate matter in wistar albino rats. It reduces the edema and let the intra-bronchial walls thin indicates the reduction in the inflammation in lung tissues.

Conflict of interest: There is no conflict of interest.

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