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Paper

PAPER

Beneficial effects of polyherbal formulation (Bronco-T) on formaldehyde-induced lung toxicity in male Wistar rats

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Abstract

Polyherbal compound (Bronco-T) has been extensively used as a traditional medicine for various therapies. However, very few report studies on anti-inflammatory and lung regeneration properties are evidenced. In the present study, we evaluated the beneficial actions and anti-inflammatory properties of polyherbal medicine, Bronco-T, exhibited by treating the lungs of rats exposed to formaldehyde to evaluate the beneficial properties. For this study, we divided into five groups': i.e. Group-I served as a control and the other four groups such as II, III, IV, and V are experimental. All animals maintained by regular feed and water *ad libitum* during the study. Formaldehyde vapors exposure at a single period of time (1 hour) daily (40%formaldehyde at room temperature) for 21 days period exposed all groups. The Bronco-T extracts about 50 mg/kg BW administered to experimental groups and group IV rats treated with 500µ grams/Kg BW salbutamol. To understand the impact of formaldehyde exposure on the beneficial effects of Bronco-T, we evaluated hematological parameters, bronchoalveolar lavage (BAL), histamine levels, and histological alterations of lung architecture. Formaldehyde-induced adverse effects in lung and increased histamine levels in BAL compared to Bronco-T-treated rats act as a preventive immunological role in blood toxicity and recovery of lung architecture in Bronco-T-treated rats. This study showed the evaluation of antihistamine levels through HPLC analysis. Bronco-T has antioxidant and anti-histamine properties as the widest therapeutic window, and we continue to evaluate the pharmacological evaluations needed in our further studies.

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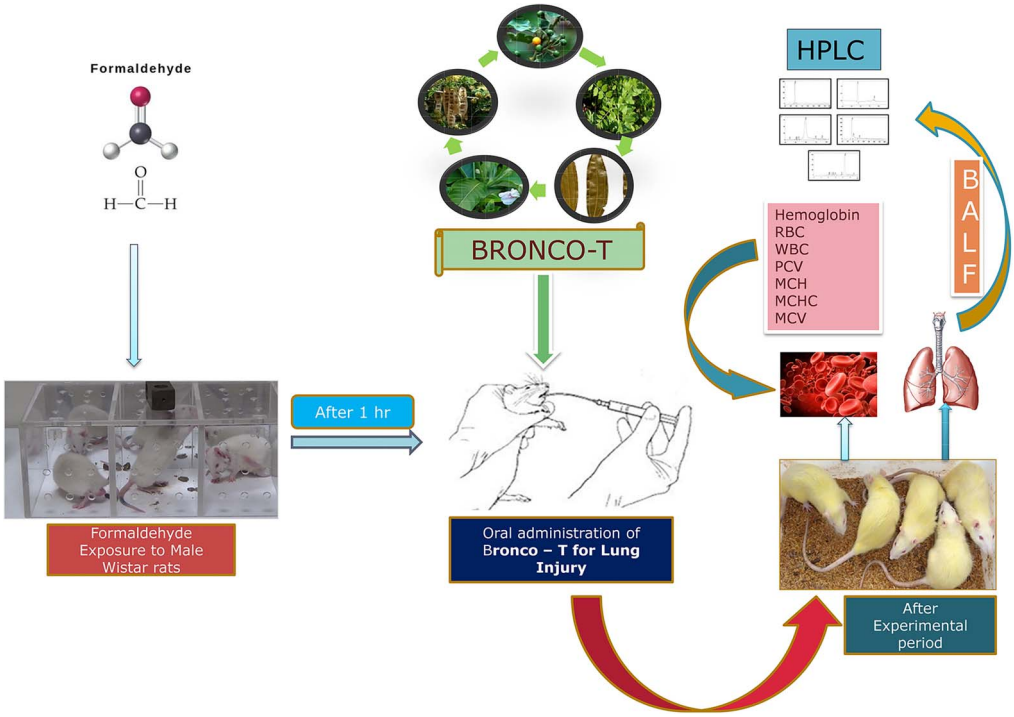
Graphical Abstract



Key words: formaldehyde, poly-herbal (Bronco-T), histamine, bronchoalveolar lavage

Introduction

The effects of formaldehyde toxicity have been widely discussed during the last few years. Formaldehyde (FA) is found in automobile engine exhausts, sterilizing agents, floor coverings, foam lagging materials, office furnishings, and cigarette side stream [1]. After FA inhalation, symptoms like asthma, as well as bronchi inflammation of bronchial tubes, are observed [2–4]. In 1990, the clean air act amendments were clearly defined formaldehyde as a noxious air pollutant composite to dogmatic action. The industries of formaldehyde holdout the initial synthesis into many other processes and few manufacturers are purely devoted to formaldehyde alone. In 2010, the US environmental protection agency (EPA) Incorporated risk information system (IRIS) outline toxicological review of FA noted that this study's findings maintain the biological plausibility of formaldehyde exposure on the hematopoietic system states that reductions in cell counts of all the blood cells. The animal studies usually reported neither hematotoxicity nor leukemia [5] associated with formaldehyde exposure. FA augmented mean cell volume and provide the best confirmation for bone marrow toxicity. Although a few studies reported that FA changes the blood profiles and hematology studies [6]. No statistically significant difference in measured formaldehyde in the blood of humans or laboratory animals exposed to relatively high levels has been observed [7, 8]. Formaldehyde can penetrate the blood stream through the respiratory tract or the GI tracts easily when exposed humans. In the blood, formaldehyde goes through a reduction–oxidation reaction and combines with macromolecules and causing irreversible cross-links makes the worse to the biological system [9,10]. Hence, the respiratory tract is the foremost targets of FA and after its exposure, cognizance of inflammatory mediator histamine [11]. The mechanisms of FA-induced asthma might be lower respiratory tract irritation from inhaled FA, immunologic reaction to FA, and/or FA-induced direct release of histamine [11]. In chronic lung disease, histamine released by degradation of the mast cells and, then they sought to observe changes in lung histamine levels in an animal model of pulmonary fibrosis [12]. Since released histamine is a marker for mast cell degranulation, assessment of bronchoalveolar lavage (BAL) fluid histamine is one means of evaluating local mast cell degranulation occurring in the airways and lungs. Number of articles revealed that FA is a pro-inflammatory agent, after inhalation causes BAL of mice, which increases the number of inflammatory cells upon allergic lung inflammation [13], and reduction of pulmonary function level.

Plant-based medicines having a great value of anti-inflammatory or immune-modulating property compounds evidenced by number of articles reported that they have a great potential to decrease the anti-inflammatory properties [14]. Now a day's, people are mostly dependent on modern medicines for instant relief from the disease, but chemical-based medicines are synthetic chemicals and most part gives relief representatively and are designed mostly for particular target receptors. Polyherbal natural medicines are promising and have fewer side effects to reduce the inflammatory source of the disease and create a healthy lifestyle at the same time to prevent the reoccurrence of inequity and support wellness, longevity, and vitality. We used polyhedral (Bronco-T), a natural herbal formula in the experiment, which contains the ingredients *Cinnamomum tamala, Albizia lebbeck, Solanum xanthocarpum, Justicia adhatoda,* and

Glycyrrhiza glabra. The plant of *Albizia lebbeck* bark especially used in the treatment of asthma, allergic disorders, and respiratory disorders are reported [15, 16]. Histamine-induced bronchospasm in guinea pig was cured by using the decoction of *Albizia lebbeck* bark and flower. Other reports state that it took an important role in protection of guinea pig against lung inflammation [17] with smooth muscle relaxation property of natural medicine when used as a therapeutic medicine. In the system of Siddha, practitioners everywhere used *Solanum xanthocarpum* medicine in southern India to cure respiratory diseases [18]. The *Solanum xanthocarpum* extract reported that it prevents against mast cell degranulation, anti-histamine and eosinophil, which mainly used for the treatment of asthma [19], and it also used for smooth muscle relaxation and antagonism of asthma mediators. The plant *Kantakari* is widely used to treat respiratory diseases in traditional medicine of Ayurveda [20]. *Cinnamon* is used as a peroxide, *p*-anisidine, thiobarbituric acid, and total carbonyl value method [21, 22]. Jestica adhatoda and madhuyashti is a well-known drug in Ayurveda and Unani medicine [23].

This present study beneficial actions of Bronco-T on lung inflammation encountered by inhaled material FA is the site of the respiratory tract are the area of our interest. 1) Hematology parameters evaluated to find the cause, prognosis, treatment, and prevention of diseases. 2) Bronchoalveolar lavage (BAL) provides a sampling of a rat body fluid that can provide valuable information on the reaction of the lung to inhaled FA materials. 3) Lung histopathology observations helped to find the inflammation in the lung in exposed rats and regained architectures in treated rat groups. Advantage of BAL analysis of lung is that one can pick up early indicators of biochemical changes leading to later morphological changes of lungs, which helps to better understand the inflammation progression of FA toxicity. A second advantage is that the BAL fluid analyses are quantitative, and measures can be obtained for the Bronco-T treatment. This work contributes to understand the effects of FA exposure in the development of lung inflammation and therapeutics properties and anti-inflammatory benefits of Bronco-T when treated to FA-exposed rats.

Materials and Methods

Animals

Thirty healthy male Wister rats aged 2–3 months, with weight between 180 and 250 g, were obtained from the breeding farm of the animal house of the University of Bangalore for this study. All rats were housed in each polycarbonate cage maintained under specific pathogen-free conditions of$21 \pm 25\ ^{\circ}C$ and humidity 45–64% with 12 hour day/night cycle for one week before use and fed with standard diet obtained from Hindustan lever Ltd., Bangalore, India, and water was available *ad libitum* for the duration of the experimental period.

Experimental design

The animals were randomly assigned into five main groups I, II, III, IV, and V containing six rats in each group ($n = 6$). Group-I corresponded to the control group and Groups-II–V to the experimental groups. The control group (Group-I) was unexposed to 40% formaldehyde as they were kept independently and Group II dealt with Bronco-T, while the other experimental animals (Group-III to Group-V) were exhibited to 40% formaldehyde vapor environs at a single period of time (1 hour) daily (40%formaldehyde at room temperature). The rats were exposed with 1 mL of 40% formaldehyde by soaking it in cotton wool and arranged in encompass (designed) wire gauze within the animal cage, thus exposing the animals to formaldehyde vapor at room temperature for 1 hour daily. The procedure was repeated for 21 days in Groups-III, IV, and V correspondingly. The treated Group-IV accompanied by Bronco-T herbal extracts was administered through oral route (using gavage intubation) with 50 mg/kg body weight. Group-V rats that were treated with 5 µg of salbutamol drug were considered to validate the synthetic drug therapeutics effects. After 1-hour exposure of FA, all animals were freed from the wire gauze within the animal cage with tolerable admittance to feed and water *ad libitum* during the study.

Hematological assessment

All the rat groups were sacrificed after the completion of experimental period; the whole blood was collected by cardiac puncture into EDTA-coated tubes. The blood samples were used for the analysis. The blood parameters analyzed include the hemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), platelet (PLT), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). For this analysis, we used automated hematology Analyzer ERMA (model pce 210).

Preparation of samples: lung BAL

In each animal, intra-tracheal instillation was performed with saline and BAL collections done as previously described with PBS (12). Samples were immediately frozen until analysis; lung BAL samples were thawed mixed and filtered (Millex 0.22 µm unit). The samples were kept at $5\ ^{\circ}C$ as recommended (22); only polyethylene plastic ware was used in preparing the samples and standards.

Bronchoalveolar lavage suction/histamine assay

Rats were anaesthetized with isoflurane; during euthanization, the chest cavity was dissected to expose the lung and the trachea. BAL fluid was taken from euthanized rats. Briefly, tracheae of rats were cannulated with PE-90 polyethylene tubing closed to a 19-gauge center of the pointer that was tenderly inserted into the trachea, and 30 mL/kg of chilled saline was administered and aspirated gradually through the needle hub. BAL fluids were centrifuged at 300 g for 5 min at $4^{\circ}C$. *Ortho*-phthaldialdehyde (OPA), histamine diphosphate, were obtained from Sigma-Aldrich, phosphate-buffered saline (PBS) was from Thermo Fisher Scientific. In this technique, we used reagent grade or better compounds. The stock solutions of OPA and histamine were prepared freshly.

Preparation of standards for HPLC

The standards of histamine BAL sample were prepared in PBS counterpart to 2–55 ng/ml and analyzed on the system as described above.

HPLC analysis

Detection of histamine by fluorimetric method using OPA in biological tissues was described in 1959 [24]. In present experiment, we used Kratos fluorescence detector (Model FS970). The HPLC was a Waters 600 multi solvent delivery system outfitted with a 700 Satellite WISP auto-injector (with refrigeration unit). Isocratic flow separations were done on a 100 × 4.6 mm I.D., 5 pm particle size, partisil5 SCX RACII column (Whatman, Clifton, NJ, USA) with 0.17 M KH_2P0_4 at a flow rate of 1 mL/min. A 250 × 4.6 mm I.D. Column elutes were mixed with alkaline OPA solution in a turbulent mixer. The OPA reprivatizing solution was prepared as described [21] and delivered to the mixer via an Eldex pump (Model A-30-S) at ca. 0.5 mL/min. Subsequently, the reaction mixture was flowed through reaction coil (PTFE tubing 305 cm × 0.5 mm I.D.). Optimal compassion was obtained when the postcolumn reaction pH was adjusted between 11.7 and 12.0. Effluent pH was monitored via a VWR pH meter model (2000) [25]. The effluent was not acidified because endogenous histamine exceeded 1 ng/ml. The OPA derived was detected using an excitation wavelength of 230 nm and a 418-nm Kratos filter.

Histological pathology investigation of Lungs

The animals were euthanized by the end of the exposure period, the thoracic cage was cut open, and random samples of the lungs from animals in each group were collected. They were preserved in 10% formalin by immersing in high volume of the fixative, processed for light microscopic study, and examined histologically. The tissues were fixed in neutralized formalin, dehydrated with ethanol, and embedded in paraffin wax (56°C). Some tissues were made into thin sections by microtome and stained with hematoxylin and eosin. The stained sections were viewed under light microscope, and the histological changes were recorded with the help of a pathologist.

Statistical analysis

Data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included two-way analysis of variance (ANOVA). A value of $P < 0.001$ and $P < 0.01$ was considered to indicate a significant difference between groups. All the data were expressed as the mean ± standard error of mean (SEM).

Results

Formaldehyde inhalation effects of on hematological parameters

Our results showed that the control and the effect of the Formaldehyde inhaled exposed rats on hematological indices, rats treated with Bronco-T and salbutamol values are presented in Tables 1 and 2. Hemoglobin, RBC were significantly lower ($P < 0.01$), in the FA exposed rats compared to the controls group rats after 21 days. In Group-II rats, the count of RBC and Hemoglobin level was slightly increased with non-significant change when compared to group-I rats. In group-III rats showing, significantly decreased, RBC count and hemoglobin levels, which were restored in the FA exposed rats treated with Bronco-T (Group-IV) better than the Salbutamol (Group-V) when compared to formaldehyde exposure rats (Group-III) (Table 1). After the experimental duration of 21 days, the WBC count, neutrophils, lymphocytes, eosinophils, basophils, monocytes were significantly higher in FA exposed rats compared to control group rats. Group-II rats neutrophils, lymphocytes, eosinophils, basophils, monocytes were slightly changed but not significant when compared to Group-I. In FA exposed rats treated with Bronco-T and salbutamol were showed decreased levels of neutrophils, lymphocytes, eosinophils, basophils and monocytes when compared to FA exposed rats.

In Table 2, a decrease in platelet count was observed only in the group exposed to FA. No changes in comparison with the control were observed in the two remaining groups of animals. In mutually IV and V Groups, which were treated, with Bronco-T and salbutamol a slight changes in the reaction intensity and pattern were detected. In the FA-inhaled group without treatment, platelet count was found to decrease. The platelet count in control rats was found to be 896.66- ± 55.73X 10^9/L. In Group-II, the platelet count was slightly decreased compared to control rats. In Group-III, the platelet count significantly decreased to 521.66 ± 36.56 X 10^9/L. Groups-IV and V rats also showed slightly decreased levels of platelet count when compared to control group rats. However, the count of platelet rise in them was increased when compared to Group-III rats. PCV was decreased in Group-III rats and increased the levels of MCH, MCHC, and MCV when compared to Group-I rats. There is no significant change in Group-II rats. Great improvement was observed in the levels of PCV and decreased the levels of MCH, MCHC, and MCV in Groups-IV and V rats compared to Group-III rats (Table 2).

Histamine validations in lung BAL HPLC analysis

The performance parameters of the proposed method were Adequate for the detection and quantification of the histamine. The combination of SCX and postcolumn derivatization with OPA provided sufficient selectivity to detect histamine in crude BAL samples, where histamine eluted at 4.8 min. Fig. 1A shows a chromatogram of control lung lavage with endogenous histamine. HPLC BAL peak identity was confirmed by co-injection of histamine standards with lavage samples. The sample volume used in this study was maintained constant at 40 µL, but injection of larger volumes (up to 60 µL) produced no change in the retention time compared to standard referenced histamine in (Fig. 1**). The histamine concentration was 0.160 ng/ml in control group. Fig. 1B shows that under the selected HPLC conditions, the retention time for histamine were 4.6 min. Slight change was seen in elution pattern of histamine when compared to control rats Group-I. Histamine levels showed minor change of 0.168 ng/ml. The retention time reproducibility was calculated from $n = 6$ analyses: the coefficient of variation (CV) was less than 4%. Fig. 1C shows that there was a greater level of histamine in the FA-exposed rats without treatment (Group-III) compared to the control rats lavages, and the histamine concentrations was found to be 2.24 ng/ml and the retention time was 4.9 min. Group-IV rats have no significant differences occurred within and among the subgroups examined. As shown in Fig. 1D, BAL histamine levels 0.171 ng/ml were significantly lower when compared to Group-III and the retention time was 4.5 min, with a similar pattern in control (Group-I) rats. Fig. 1E shows retention time for histamine were 4.1 min for rats exposed to FA and those treated with salbutamol had a lower histamine level of 0.179 ng/ml compared to Group-III rats, but had a lower improvement compared to Group-IV.

Lung histology observations

In present experiment, Group-I (control) rats showed Fig. 2A standard pulmonary tissue architecture, folded columnar epithelial cells of bronchiole with clear obvious bronchial passages and

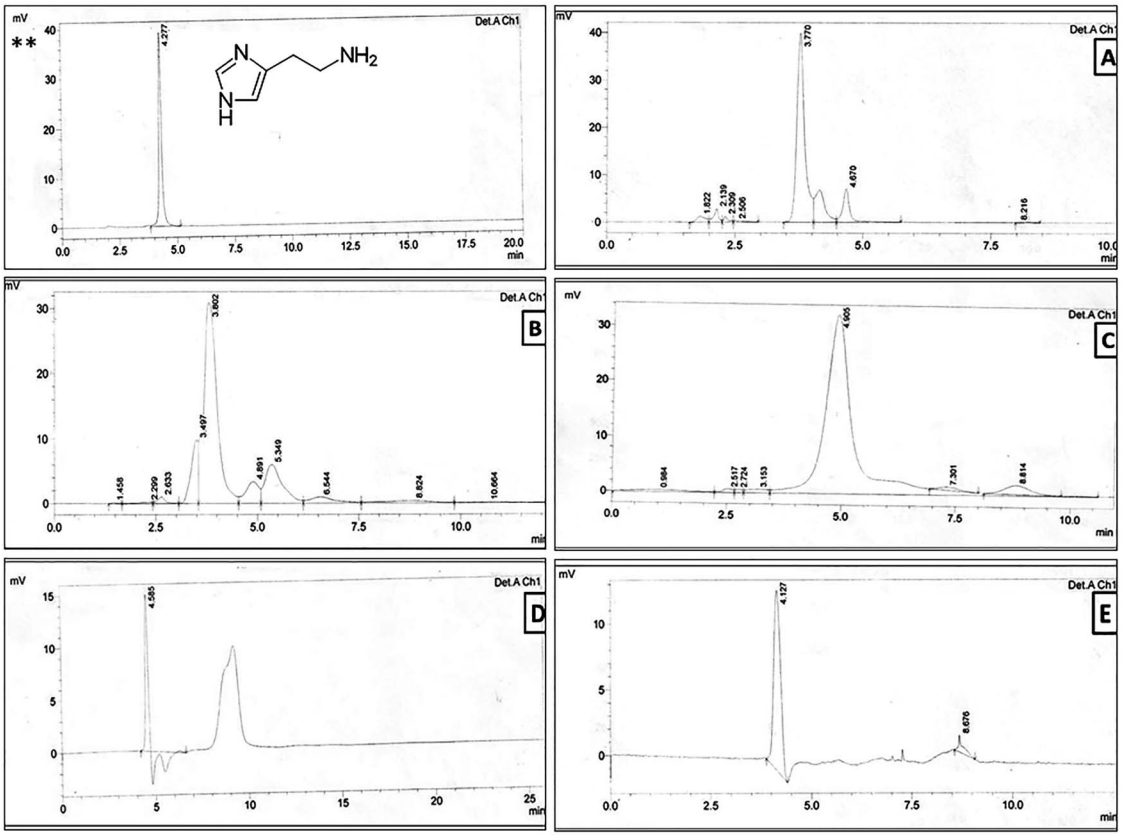


Figure 1: Chromatogram of a BAL sample from control and experimental rats **Chromatogram of a reference sample histamine eluted at 4.2 min. (**A**). Chromatogram of a branchoalveolar lavage sample from Group-I rats showing endogenous histamine eluted at 4.8 min. (**B**). Chromatogram of a branchoalveolar lavage sample from Group-II rats showing endogenous histamine eluted at 4.6 min (**C**). Chromatogram of a branchoalveolar lavage sample from Group-III rats showing endogenous histamine eluted at 4.9 min (**D**). Chromatogram of a branchoalveolar lavage sample from Group-IV rats showing endogenous histamine eluted at 4.5 min (**E**). Chromatogram of a branchoalveolar lavage sample from Group-V rats showing endogenous histamine eluted at 4.1 min. (**F**). Chromatogram of a branchoalveolar lavage sample from Group-V: a bronchoalveolar lavage sample showing endogenous histamine eluted at 4.1 min.

alveolar cavities as well as the alveolar sacs, thin interalveolar septa, and the alveoli. Two types of epithelial cells (pneumocytes), squamous epithelial cells (pneumocyte type I), and large cuboidal cells (pneumocyte Type II) were presented in the alveolar. Alveolar septal blood capillaries were standard width and no abnormality in alveolar septa. The standard para-bronchiolar lymphoid aggregation and normal construction of bronchiolar epithelium was observed.

In Group-II, rats showed Fig. 2B the alveolar septa had normal thickness with no abnormality in alveolar septal blood capillaries. There is no change in alveolar wall, with normal architecture of smooth muscle and tubular cells in the lungs. When compared to Group-I rats, no significant change was observed. The lung section of Group-III rats exhibited (Fig. 2C) different signs of damage. Alveolar hemorrhage/edema, inflammatory cell infiltration, bronchiolar epithelia degeneration, alveolar wall disruptions, and hyperplasia in smooth cell organelles were also observed. The Groups-IV and V animals on treatment with Bronco-T and Salbutamol to the formaldehyde exposed rats Fig. 2 D and E better development in lung epithelial layers and decreased inflammation in the alveolar surface had normal microvilli. Epithelial dysplasia also was cleared. However, Group-V (treated with Salbutamol) rats are poor recover compared with Group-IV (treated with Bronco-T) and the rats are exhibited better to regain of the smashed pulmonary cells.

Discussion

In toxicology, studies of inhaled FA chemical agent and the degree of an induced inflammatory response and the time course of recovery from or intensification of the process are not properly understood. While FA chemicals contribute to the stimulation of asthma in the general population, the underlying molecular pathogenesis of this relationship is not yet well understood. The airway reactivity, lung histological changes, histamine secretion in the lungs, and FA-exposed rats significantly contribute to the better understanding of FA exposures. FA-exposed rat lungs can be recovered by treating with Brancho-T polyherbal and antihistamine property-based therapeutics that will provide possible, on the toxicity of FA exposure data to public health. This study reports hematological data, analysis of lungs BAL HPLC analysis, therapeutic treatment of Bronco-T validations, and histopathology observations of lunch tissues. Initially, we validated hematological analysis.

Blood can act as a pathological and physiological pointer of animal health [26]. Each of the blood count parameters, specifically, white blood cell (WBC) count and its component levels such as neutrophils, lymphocytes, eosinophils, basophils, monocytes, red blood cell (RBC) count, and its component hemoglobin and platelets along with mean corpuscular volume MCV, MCH, MCHC, and PCV was examined as the primary outcome variables of interest. Results as reported in [27] at disease conditions,

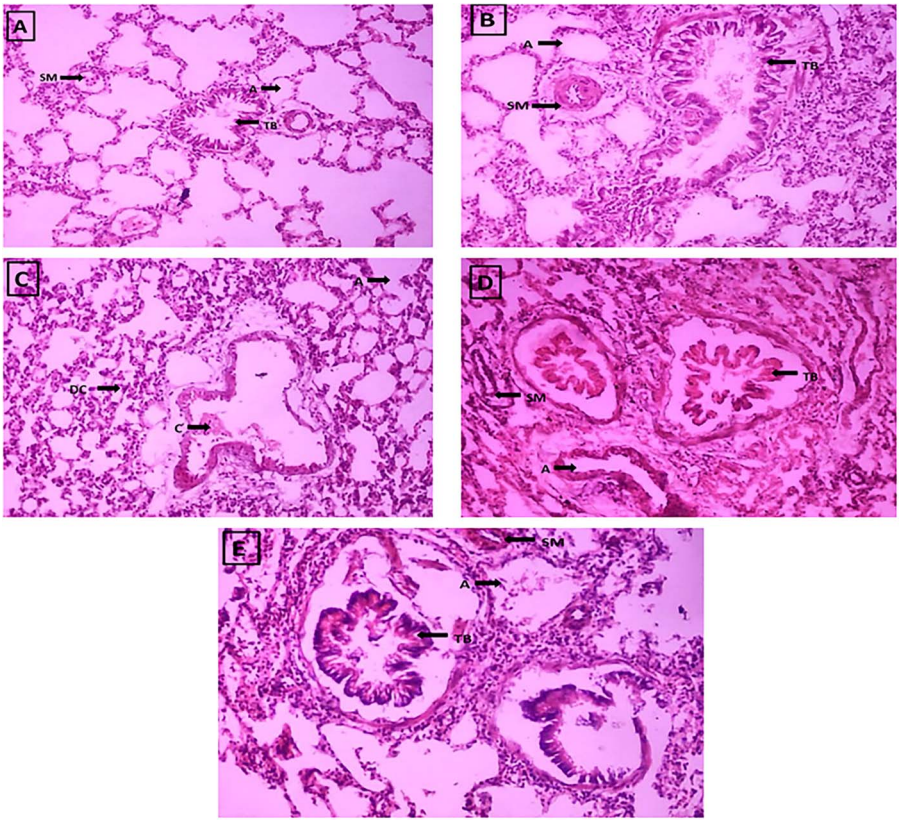


Figure 2: Histology photographs of control and experimental groups of rats lungs. **(A)** Normal structural design in the lung tissue of control rats: alveoli (A), smooth muscle (SM), terminal bronchiole (TB) (10X magnification). **(B)** Normal architecture of alveoli (A), smooth muscle (SM), terminal bronchiole (TB). in lung tissue on treatment with Bronco-T (10X magnification). **(C)** Photograph shows alveoli (A) congestion (C), degenerative changes (DC) on expose to formaldehyde (10X magnification). **(D)** Regenerative changes in lung was observed similar to normal architecture of lung on treatment with Bronco-T (10X magnification) alveoli (A), smooth muscle (SM), terminal bronchiole (TB). **(E)** Regenerative changes of lung tissue similar to normal architecture of lung on treatment with salbutamol (10X magnification) alveoli (A), smooth muscle (SM), terminal bronchiole (TB)

hematological parameters were affected resembling hematopoietic physiology and due to immunological retort. FA-exposed rats' blood has lower count of RBC than control group rats; this type of condition in blood is called anemia. Red blood cells may also have less hemoglobin than control rats. Red blood cells carry oxygen from lungs to throughout the body because it provides iron-rich protein called hemoglobin. Our body needs oxygen to work properly. With less quantity of red blood cells or less hemoglobin and the fall in the levels of the body iron content, our body may not acquire sufficient oxygen. The presence of circulating immune complexes thus may result in a serum sickness like reaction. While these mechanisms are largely theoretic, cases of autoimmune hemolytic anemia associated with large exposures have been reported [28, 29]. For instance, allergic affliction might affect the hematological parameters together with eosinophils and neutrophils [30, 31]. In-Group-III rats have significantly increased count of WBCs compared with Group-I. At the condition of inflammation, airways converted to be narrower where smaller amount of air can pass through them; less oxygen reaching the tissue is called hypoxia or hypoxemia (GINA 2015). WBCs, mainly neutrophils, basophils, and eosinophils, play a key role in pathologic condition of allergic afflictions [32,33]. On the contrary, an exacerbated eosinophilic reaction was observed when allergen and FA exposures occurred simultaneously [34] While the Groups-IV and V showed decrease in the number of WBCs compared with FA exposure without treated rats in Group-IV rats, WBC count values observed were better than salbutamol-treated group.

Platelet count and PCV were decreased whereas MCV, MCH, and MCHC were highly increased in Group-III rats when compared to control group. Red blood cell indices reflect the size of MCV and level of hemoglobin content (MCH and MCHC) of the red blood cells and aid in the diagnosis of the cause of anemia. Increase of MCV clearly indicates the exposure to formalin-induced swelling of the erythrocytes of the rats. The values of MCV, MCH, and MCHC suggest that normocytic, normochromic anemia may have occurred in Group-III rats. The rats of Group-IV treated with Bronco-T significantly recovered the blood parameters as reported in Tables 1 and 2.

Surface release/accumulation of cellular secretary products and effect of toxicant alterations reliability of pulmonary epithelial and cell damages in lungs are extensively evaluated by using bronchoalveolar lavage (BAL). It was also used to study the lower respiratory tract immunity in various animal models [34,35]. Lavage of more distal areas of the lung was reported as a therapy for septic lung disease and pulmonary alveolar proteinases four decades later [36, 37]. Released histamine is a marker for mast cell degranulation and a potent inflammatory mediator, commonly associated with allergic reactions, promoting vascular and tissue changes and possessing high chemoattractant activity. Measurement of histamine in BAL is one avenue of assessing local mast cell destruction occurring in the lungs. Histamine is

Table 1: Blood parameters of control, FA-exposed and treated groups

	Group-I (Control)	Group-II (Control + Bronco-T)	Group-III (FA exposed)	Group-IV (FA exposed + Bronco-T)	Group-V (FA exposed + Salbutamol)
Hemoglobin (g/dL)	12.6 ± 0.75	13.4 ± 0.67 (+6.34) **NS**	9.8 ± 0.52(−22.22) *$P < 0.001$	12.28 ± 0.57(−2.53) **NS**	11.09 ± 0.57 (−11.98) $P < 0.01$
Total RBC Count (10^{12}/L)	2.97 ± 0.27	3.16 ± 0.38 (+6.39) **NS**	1.53 ± 0.13 (−48.48) *$P < 0.001$	2.64 ± 0.25 (−11.11) $P < 0.01$	2.44 ± 0.25 (−17.84) $P < 0.001$
Total WBC (10^9/L)	61.40 ± 3.57	62.80 ± 3.03 (+1.95) **NS**	133.20 ± 7.01 (+116.93) *$P < 0.001$	63.40 ± 4.77 (+3.26) $P < 0.01$	69.20 ± 2.38 (+12.7) $P < 0.001$
Neutrophils %	42.83 ± 4.83	42.66 ± 4.32 (−0.39) **NS**	75.5 ± 7.41 (+76.27) *$P < 0.001$	46.33 ± 4.57 (+8.17) **NS**	56 ± 3.84 (+30.74) $P < 0.001$
Lymphocytes %	44 ± 3.74	42.5 ± 3.88 (−3.4) **NS**	65.5 ± 6.04 (+71.59) *$P < 0.001$	44.83 ± 4.34 (+1.8)	58.5 ± 5.61 (+32.95) $P < 0.001$
Eosinophils %	1.66 ± 0.81	1.83 ± 0.75 (+10.24)	5.66 ± 1.01 (+240.96) *$P < 0.001$	2.01 ± 0.89 (+20.48) **NS**	2.83 ± 0.75 (+4.48) $P < 0.01$
Basophils %	0.40 ± 0.01	0.4 ± 0.02 (+0.60) **NS**	1.2 ± 0.08 (−200) *$P < 0.001$	0.43 ± 0.02 (+7.50) $P < 0.01$	0.5 ± 0.03 (+25.00) $P < 0.001$
Monocytes %	2.33 ± 1.03	2.16 ± 1.16 (−11.58) **NS**	9.50 ± 0.9 (+307.72) *$P < 0.001$	2.50 ± 1.04 (+7.29) $P < 0.01$	2.70 ± 1.04 (+15.87) $P < 0.001$

Results represents mean ± S.D. of each group ($n = 6$)
Values are significantly different from control at $P < 0.01$and $P < 0.001$;
*indicates significant change.
NS indicates no significant change.

Table 2: Blood parameters of control, formaldehyde (FA) exposed and treated groups

	Group-I (Control)	Group-II (Control + Bronco-T)	Group-III (FA exposed)	Group-IV (FA exposed + Bronco-T)	Group-V (FA exposed + Salbutamol)
Platelet Count (10^9/L)	896.66 ± 55.73	885 ± 30.82 (−1.3) $P < 0.01$	521.66 ± 36.56 (−41.82) *$P < 0.001$	893.33 ± 44.2 7 (−0.37) **NS**	890 ± 34.05 (−0.74) **NS**
PCV (%)	30.00 ± 3.63	30.16 ± 3.63 (−0.5) **NS**	13.5 ± 1.87 (−55.00) *$P < 0.001$	29.66 ± 2.36 (−1.13) NS	27.53 ± 2.74 (−8.20) NS
MCH (pg)	4.33 ± 0.47	4.25 ± 0.47 (−1.84) **NS**	6.88 ± 0.46 (+58.89) *$P < 0.001$	4.55 ± 0.59 (+5.01) **NS**	5.27 ± 0.58 (+21.70) $P < 0.01$
MCHC (g/dl)	1.29 ± 0.11	1.31 ± 0.13 (+1.5) **NS**	3.46 ± 0.24 (+168.21) *$P < 0.001$	1.35 ± 0.10 (+4.65) **NS**	1.47 ± 0.10 (+13.95) $P < 0.01$
MCV (FL)	13.75 ± 0.11	12.92 ± 0.11 (−6.03) **NS**	27.72 ± 1.01 (+101.60) *$P < 0.001$	14.17 ± 1.11 (+3.05) **NS**	16.99 ± 1.13 (+23.56) $P < 0.001$

Results represents mean ± S.D. of each group ($n = 6$)
Values are significantly different from control at $P < 0.01$and $P < 0.01$;
*indicates significant change.
NS indicates no significant change.

a vasoactive amine that plays an important role in the early acute inflammatory response. It is stored in the granules of mast cells, basophils, and platelets. Histamine research in BAL is an attractive perspective for the potential therapeutic exploitation of new drug targets. The intention of the present study was to determine the amount of histamine levels in BAL fluid from FA-exposed rats and how the lungs got inflamed caused by the FA exposure and how the lungs recovered when treated with the broncho-T. Our findings indicate that the FA-inhaled rats have higher BAL histamine levels than do the normal controls. Furthermore, higher BAL histamine levels were associated with parameters that have been suggested by other studies to be associated with more active lung inflammation and/or a worse prognosis. Histamine and acting on H1-receptors are playing key role in contraction of uterus. Smooth muscle of the ileum, bronchi, and bronchioles [38]. Histamine-induced bronchiolar constriction has been concerned in the first phase of bronchial asthma [39]. Histamine-mediated inflammatory reactions amplify the inflammation to cell sites and may errand the establishment of a chronic inflammatory response. An increased amount of histamine is found in bronchoalveolar lavage, reaching a concentration of 2.8 ng/ml after allergic reactions in asthmatic patients [40]. In present study, normal controls (Group-I) and Group-II rats found to have little or no detectable amounts of histamine in BAL. In contrast, Group-III rats had much higher BAL fluid histamine levels than did the control rats. Group-IV rats with Bronco-T were found to have decreased levels of histamine compare to Group-III rats. Bronco-T may not only reduce FA toxic action but also decrease their retention in both primary and secondary deposition sites and thus attenuate their subchronic and chronic toxicity on the organ–systemic–organism levels. Bronco-T inhibitory effect is greater than that observed with salbutamol treatment (Group-V).

In the present work, we report the effects of FA exposure on the outcome of allergic lung response in rats, notably on the respiratory mechanics and beneficiary effects of Bronco-T in lung injury. We have used FA, an indoor and outdoor noxious waste, widely working in many industries and laboratories [41] to investigate the influence of pollutants on the development of allergic lung disease. Consequently, the respiratory tract is one of the most important targets of FA and later than its exposure, an immune reaction with inflammatory intermediary's release, including histamine, is observed [11]. Histamine-induced vascular permeability is an H_1-mediated effect that is caused by contraction of actomyosin fibers in endothelial cells of the postcapillary venues [42]. Improved permeability occurs in all allergic diseases and therefore is a feature of allergic rhinitis, asthma, urticaria, and anaphylaxis. Histamine's ability to induce smooth muscle contraction forms the basis for the histamine bioassay. In this experiment, Bronco-T may enhance the curative effect in BAL fluid in the lungs. This suggests that the Bronco-T may possess antihistamine activity against FA-induced inflammatory diseases. Decreased levels of the histamine levels in Group-IV when compared to other control groups were noted. With regard to the affiliation between formaldehyde exposure and allergic diseases, occupational asthma due to formaldehyde exposure has been reported [43, 44]. The underlying mechanisms of the induction of airway inflammation and hyper-reactivity are complex. Therefore, our findings that a low concentration of formaldehyde affects mast cell functions may provide useful clues.

In the present study, the histological and the toxicological effect of formaldehyde exposure on structure and function of rat lungs was under taken (Fig. 2 A–E). An infection in the airway would release histamine from mast cells, and that would be one of the reasons. The lungs take an important role in congregation protection and regulation of circulating levels of biologically active supplies by extensive surface of pulmonary vascular cradle [45]. Respiratory tract and lungs were first organs affected by inhalation of formaldehyde, which makes the target organ of airborne pollutants [46–48]. In Group-III rats, exposure of 40% formaldehyde mechanism brought about the ulceration of alveoli was excavation of the surface epithelium and sustaining tissues of the alveolar wall. The current study investigated that inhalation of concentrated formaldehyde for 1 hour/day (21 days) lead to bronchiolar epithelia degeneration, alveolar wall disruptions, alveolar hemorrhage/edema, inflammatory cell infiltration, and hyperplasia of smooth cell organelles. Some specimens showed signs of epithelial dysplasia from earlier studies, which is in agreement with these findings [49], and also detected in rats [50]. Our data might claim in favor that FA exposure of rats could cause lung injury. Most previously published studies observing histamine release from pulmonary mast cells have used partially purified enzymatically digested and dispersed lung cells [51,52]. The rats treated with Bronco-T significantly diminished inflammation by reducing the inflammatory cell infiltration in lung tissue alveolar surface that had normal microvilli and recovered alveolar walls. Group-V rats also make progress in the alterations observed due to formaldehyde inhalation rats. However, these rats are when compared with Group-IV (treated with Bronco-T) showed slower regain of the damaged cells in lungs. Notably, administration of Bronco-T involved fast-accelerated alterations indicating that the anti-inflammatory improved mechanism against formaldehyde inhalation. Our results provide some plant-based biomedicine properties of Bronco-T, which is acknowledged by the effective anti-inflammatory properties. The Bronco-T decreases the histamine levels, which can be used for bronchitis and respiratory abnormalities; the lung architecture of histopathological observations also evidenced with recovered bronchi is greatly caused by chemical (FA)-induced toxicity. This Bronco-T greatly helps to recover from the lung damage caused by the FA and maybe further in detailed studies much needed to understand the lung inflammatory diseases and therapeutic biomedicine properties of Bronco-T.

Conclusions

Findings from this study highlighted the presence of a protective mechanism of Bronco-T against toxicity of FA-inhaled rats. Bronco-T has effectively reduced the histamine levels in BAL of FA-exposed rats. The better improvement in blood hematological parameters following withdrawal in FA-exposed rats observed when treated with Bronco-T compared to Salbutamol-treated rats group. The present work investigations suggest that polyherbal compound Bronco-T showing the anti-histamine properties administered after FA inhalation. This polyherbal Bronco-T helped to treat symptoms of lung inflammation caused by the FA. This studies also helped to investigate bronchitis, asthma if they are to be getting better relief and benefit in lung-infection-related therapeutic studies. Therefore, Bronco-T has anti-histamine properties that may open to widest therapeutic window for lung therapeutic drugs. However, formal polyherbal formulation Bronco-T have greatest advantage in the treatment inhaled lung injury with which is natural poly herbal drug with less side effects are expected more reliable than the synthetic drugs. Our future research projects expand in more details about this formulation and biomedicine properties dose dependent

investigations to find the therapeutic mechanisms and natural compound potent properties of Bronco-T and efficacy studies in lung-based therapeutic drugs.

Conflicts of Interest

The authors declare no conflict of interest.

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