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EVALUATION OF A POLYHERBAL FORMULATION IN EXPERIMENTALLY INDUCED ASTHMA IN SPRAGUE DAWLEY RATS

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ABSTRACT

Asthma is defined as an airway hyperresponsiveness which is basically brought on by chronic inflammation which causes repeated events of wheeziness, breathless-ness, stiffness in chest, and/or coughing that might change over time and it's concentration might also change with time. Various epidemiological study it is found that asthma is one the utmost widespread long-lasting disease with high morbidity and low mortality rate. There are various medications for the treatment; leukotriene modifiers, corticosteroids, β adrenergic agonists, anti-cholinergics, and others, but these give us just symptomatic effects and these also have various side or adverse effects. Whereas, variety of medications from, various plant sources have been studied for their potential anti-asthmatic properties in the course of past periods of years with little to no adverse effects. The target of the investigation is to show the efficacy of Polyherbal formulation acting through different mechanism of action and show its effect on experimentally induced asthma. Here, Grouping of 5 which 6 animal (Sprague Dawley Rat) in each group, i.e. Normal Control, Disease Control, Standard Control, Low Dose of Polyherbal formulation and High Dose of Polyherbal formulation based on Acute Oral Toxicity. From this study, it is observed that low dose of Polyherbal Formulation in comparison to Standard Control (Montelukast sodium) is showing better efficacy by detection of various biochemical parameters and histopathological parameters of every group.

Keywords: Asthma, Wheezing, Epidemiology, Montelukast sodium, Polyherbal Formulation

INTRODUCTION

You frequently take your ability to breathe easily for granted. Many asthmatics worry constantly about losing control over their breathing. According to WHO report from 2021 claims that fifteen to twenty millions of people in India, including individual of different age group, are asthmatic **[1]**. Prevalent inflammatory illness of the airways known as asthma is connected to transient bouts of airway smooth muscle hyperreactivity **[2]**. Airway hyperresponsiveness brought on by chronic inflammation causes repetitive occurrences of wheeziness, breathlessness, chest stiffness, and/or coughing whose intensity changes over a period **[3]**.

When tracheobronchial smooth muscle is oversensitive to various stimuli, it narrows the airways and is frequently accompanied by increased secretion, mucosal edema, and mucus clogging **[4]**. Utmost prevalent long-lasting disease among children, it is a severe NCD that affects both children and adults. Asthma symptoms, which includes coughing, wheeziness, breathlessness, and tightness in the chest, which is caused due to inflammation and restriction of the tiny airways in the lungs **[5]**.

Person during asthma attack shows 3 possible outcomes:

1. Bronchospasm: It is a temporary narrowing of the bronchi (airways entering the lungs) **[6]**.

2. Inflammation: All areas of the airways are affected by the inflammation, including the nose and upper respiratory tract **[7]**.

3. Mucus production: Asthmatic patient's lung produce excess mucus hence, the passage of air gets blocked **[8]**.

Asthma was rated 34th among the top causes of disease burden in 2019 according to GBD, accounting for a fifth of all disability adjusted life years (DALYs) from chronic respiratory disorders. Asthma was predicted to be responsible for 21.6 million DALYs across all age groups worldwide. The 10.2 million YLD were responsible for over half of the asthma burden **[9]**. In 2019, asthma was the 24th-leading cause of YLD worldwide, with a slightly larger burden in men than in women **[9]**. Since 2000, both hospitalization count and death rates have decreased, according to a comparison of age-standardized rates of hospitalization and moratlity rate in 24 European nations that reported both measurements between 2001 and 2005 and between 2011 and 2015 **[9]**. Compared to admission rates, death rates decreased more than they did, but there was no correlation between these national patterns **[9]**. Despite the fact that asthma mortality rates have decreased in many nations, preventable causes still contribute to the majority of asthma deaths

[9]. Nearly half of the 195 death that occurred in the U.K. at 2012 and 2013 period were untreated or received no emergency medical care, according to an extensive examination of the deaths **[9]**. Furthermore, the majority did not receive specialised medical care in the year before death **[9]**. There was proof that too many short-acting relief medications were prescribed, not enough preventer medications were prescribed, and too many long-acting 2-agonist (LABA) bronchodilator inhalers were prescribed as the only type of treatment **[9]**. A personal asthma action plan, which is known to enhance asthma management, was given to only 25% of patients. These findings, which come from an urbanised society with a history of treatment having been used since long time and a national health service that is costs no money at the point, suggest that lowering asthma mortality globally should include a major focus on increasing access to appropriate asthma medications and emergency care **[9]**.

Asthma can be caused by indoor and outdoor allergens, environmental factors like poisons, gases, smoke etc., respiratory tract infections like respiratory syncytial virus (RSV) and genetics. Triggering factors of asthma includes exercise, air pollution, dust mites, mold, pests, pets, tobacco smoke, drugs and extreme emotional expressions **[10]**. These asthma causing agents and triggering factors lead to release of various cytokines such as IL-4, IL-5 and Il-13 **[11]**. Chemokine receptor 3 (CCR3) and other chemoattractant agents, such as mast cell-derived prostaglandin D2 (PGD2) eosinophilic recruitment in the mucosa, are induced by chemokines such eotoxin 1, 2, and 3. In addition, IL-4 and 13 induce B lymphocytes to produce allergen-specific IgE, which binds to high affinity mast cell receptors and results in a reaction **[12]**. Innate lymphoid cells (ILC2) produce IL-5 and IL-13 in response to PGD2, TSLP, IL-25, and IL-33 in non-allergic eosinophilic asthma as a result of airway epithelium damage brought on by pathogens and pollutants **[12]**. The eosinophilic inflammatory response, allergies, and asthmatic remodelling are all influenced by ILC2 and Th2 cells, which are a substantial source of type 2 cytokines **[13-14]**. In comparison to individuals with mild asthma, chronic asthmatic individuals had higher levels of the circulating and sputum ILC2s that produce IL-5 and IL-13 **[15]**. Additionally, after exposing asthma patients to allergens, more ILC2s that produce IL-5 and IL-13 were discovered in their mucus **[16]**. ILC2 and Th2 cells that express IL-13 are also in charge of asthma patients' bronchial epithelial tight junction barrier leakiness **[17-18]**. Chronic inflammation, a defining feature of severe asthma, causes tissue remodelling that results in fixed

airway blockage and little response to bronchodilator therapy **[19]**. The development of airway remodelling appears to be heavily influenced by chronic persistent inflammation and the release of several cytokines, chemokines, and growth factors from inflammatory and epithelial cells **[20]**.

Asthma is induced in rat by using Ovaalbumin(OVA). 0.66ml of a suspension of 1mg OVA+ 300mg Aluminium hydroxide is given by i.p. route on Day 0 , then the same suspension was given by s.c. route on Day 8 and finally on Day 28 the rats will be challenged with 1% OVA for 15mins **[21]**. This creates anti-OVA IgE antibodies during sensitization period, these antibodies then attach to IgE receptors on mast cells. Then on challenging through i.n. causes OVA cross-linked IgE on mast cells and culminating in degranulating mast cells. Hence rat exhibits asthmatic symptoms **[22]**.

Asthma is treated by using polyherbal formulation & it consists of following herbs:

Mangifera indica leaf extract which acts on Th1/Th2 cytokines by following four different mechanism: reducing eosinophil and total inflammatory cell infiltration, lowering PGD2 in BALF and ovalbumin specific IgE in serum, reducing, Th2 related cytokines like IL-3, 4,5,9,13,17, TNF-α and increased Th1 related cytokines (INF, IL-2, 10,12) **[23]**. *Boswellia serrate* extract acts by inhibition of leukocyte elastase, inhibition of C2 convertase, inhibition of 5-LOX **[24, 25]**.

The motive of project is to do the evaluation of low &high dose of polyherbal formulation (dose calculated based on Acute oral toxicity study) against standard Montelukast sodium tablet in experimentally caused liver fibrosis in albino wistar rats. Fibrosis of liver is introduce by Tetracycline by oral route in rats. The duration of induction is of 7 days. The treatment is of duration of 28 days.

MATERIAL AND METHOD

Herbs & excipients

Boswellia serrate (Indian frankincense) extract was procured from HealthyHey Nutrition, Mumbai, India. *Mangifera indica* (Mango) leaf extract was procured from Amsar Private Limited, Indore, India. Egg Albumen (Ova-albumin) powder was procured from Qualikems Fine Chemicals Pvt.Ltd, New Delhi, India Aluminium hydroxide powder was procured from Krishna-Chem Industry, Vadodara, India. Carboxymethyl cellulose (CMC) powder was procured from Central Drug House (P) Ltd, Dahej, India

Drugs

Montelukast sodium was procured from Vishal Chemist Tower, Vadodara, India

Phytochemical Investigation

These were carried out for extract of *Boswellia serrate* and *Mangifera indica* leaves to check presence of various phytochemical constituents. Various tests such as Dragendroff test, Mayer's test, Hager's test, Wagner's test, Molisch's test, Fehling's test, Benedict's test, Shinoda test, Legal test, Keller-killiani test, Foam test, Iodine test, Tannic acid test, Tannin's test by 5% $Fecl_3$, Tannin's test by Lead acetate, Tannin's test by Acetic acid, Tannin's test by Dilute HNO_3, Salkowski test, Liebermann-Burchard test, Terpenoid test by $CHCl_3$, Volatile oil test by tip of filter paper, Volatile oil test by Characteristics aroma, Bromine test, Coumarin Glycoside test by 10% NaoH, $FeCL_3$ test, Ninhydrin test, Million's test, Biuret's test and Gelatin test **[26]**.

Preparation of polyherbal suspension

Polyherbal formulation was administered to Sprague Dawley rats in the form of **suspension.** Suspension was administered by the **oral route** of administration. The formulae for preparing a suspension of extracts of *Boswellia serrate* extract and *Mangifera indica* leaves extract were taken in the ratio of 1:1. The suspension was prepared by using various bioactive extracts of selected plant materials trituration method in mortar and pestle by using the suitable suspending agent of carboxy methyl cellulose (CMC) in the concentration of 0.5% w/v with continuous triturating. Lastly, to get a uniform product, the final volume was increased by adding pure water through continuous trituration in suspension **[27]**.

Evaluation Parameters of Suspension

Redispersibility

A measuring cylinder was used to wait for the suspension to settle. The mouth of the cylinder was closed, inverted 180 degrees, and the number of inversions needed to restore a homogenous suspension was computed.

Rheology

The apparent viscosity was used to calculate how long it took for each suspension sample to flow through a 10 ml pipette.

Flow rate = Volume of Pipette (ml)/ Flow rates of (seconds)

Particles size analysis

A key factor in the stability of suspension is the distribution of particle sizes. Optical microscopy was used to measure the particle size distribution in diluted samples.

pH

Using a Elico LI 610 pH metre, the suspension's pH was determined.

Sedimentation volume

The sedimentation volume is determined by dividing the final sediment height by the initial height of the entire suspension when the suspension settles in a cylinder under proper standard circumstances. It was

measured by noting that the sediment's volume is indicated as ultimate height and waiting a predetermined period of time to disrupt a measured volume of suspension in a graduated cylinder.

Viscosity

Viscosity was measure using Labmaan LMDV 60 Brookfield's Viscometer **[17]**.

Animals

Healthy Sprague Dawley rats (Female) of 250-300g weight were used for the study. **All of the studies and procedures reported in this study were received approval by the Institutional Animal Ethics Committee (IAEC) of Pharmacology department, Parul institute of pharmacy and research and with permission from Committee for the Control and Supervision of Experiments on Animals (CCSEA). Protocol No. PIPR 984/2022/02/12.Permitted Animals: 36 Animals were procured from Torrent Pharmaceuticals, Ahmedabad**

Housing

Sprague Dawley rats were allowed for acclimatization for seven days on pelleted standard rat food with water and housed in a group of 3 rats per cage under well-controlled standard conditions of temperature (22±3ºC), humidity (30%-70%) and 12hrs light conditions and 12hrs dark condition cycle in animal house. Animals were given fed regularly with R.O. drinking water through polypropylene water bottles with SS spout ad libitum.

Acute oral toxicity study

OECD-423 (Acute Oral Toxicity-Acute Toxic Class Method) was done on Sprague Dawley rats to evaluate the oral toxicity dose of PHF as well for determining the highest & lowest doses of PHF for treatment. As per the guideline, three female rats were administered a single starting dose of 2000mg/kg, and were observed for signs of toxicity and mortality. Animals were respectively monitored after dose administration at least once in the first 30 minutes, at regular intervals for the first 24 hours, with extra care taken in the first four hours, and daily thereafter, for a total of 14 days i.e., 2 weeks. After 14 days of daily observation animals were found to be normal. Animals were humanely sacrificed after the study was finished. Blood parameters were carried out & histopathology of the liver & kidney was done.

After the initial acute oral toxicity study, confirmatory study was also done in the same manner as of initial study **[28]**.

Animal Groupings

Protocol for evaluation of polyherbal formulation for treatment of liver fibrosis. Animals were divided into 5 groups of six animals each.

Normal Control Group (NC)

Group-1: Administered with vehicle for 28 days (p.o.) & were given access to food &

drinking water.

Disease Control Group (DC)

Group-2: Each animal was given 0.66ml of allergen suspension containing ova – albumin (1 mg) and Al(OH)3 (300mg) in saline through i.p. route and s.c. route on Day 0 and Day 8 respectively. Then challenge i.n. with 1% ova on Day 28.

Standard Control Group (SC)

Group-3: Sensitized rat was administered with 2 mg/kg of Montelukast sodium daily through oral route for 28 days.

Test Group Polyherbal Low Dose (PHF-L)

Group-4: Sensitized rat was administered with lower dose (200mg/kg) of test substance orally for 28 days.

Test Group Polyherbal High Dose (PHF-H)

Group-5: Sensitized rat was administered with higher dose (400mg/kg) of test substance orally for 28days.

Induction procedure

Female Sprague Dawley rats weighing between 220-250g was selected. Animals were assimilated to laboratory condition for the period of 14 days. Rat was induced by administering, 0.66ml of a suspension of 1mg OVA+ 300mg Aluminium hydroxide is given by i.p. route on Day 0, then the same suspension was given by s.c. route on Day 8 and finally on Day 28 the rats will be challenged with 1% OVA for 15mins **[21]**. At the end of dosing of this induction agent various liver parameters were evaluated as well as histopathological evaluation of liver was also to carried at the end of experiment by sacrificing the animals [21].

Evaluation of polyherbal formulation

Polyherbal formulation (PHF) efficacy was evaluated on the Sprague Dawley rats. PHF was administered to two different groups of Rats. PHF was given at low dose and at highest dose in two different group for the period of 28 days. Similarly standard drug i.e., Montelukast sodium was also administered at dose of 2mg/kg for the period of 28 days in SC group. Along with this, normal control group was given with 0.5%w/v CMC for 28 days through oral route and Disease control group was administered with 0.66ml of a suspension of 1mg OVA+ 300mg Aluminium hydroxide is given by i.p. route on Day 0 , then the same suspension was given by s.c. route on Day 8 and finally on Day 28 the rats will be challenged with 1% OVA for 15mins.

Blood collection

Blood was collected via retro-orbital route. The amount of blood collected was up to 1.5 ml. The blood was collected in Citrate tubes, EDTA tubes, Eppendorf tubes. The blood was collected on 29th day.

Bronchoalveolar Lavage Fluid Collection

Animals were sacrificed to obtain the bronchoalveolar lavage fluid. The BALF

was collected by following procedure: Rat was euthanized by i.p. injection of phenobarbitone sodium. Then animal was placed on it's back and 70% ethanol was sprayed on the neck. Then a scalpel was used to make an incision in the skin of the neck near the trachea. To reveal the trachea, the muscles surrounding it were cut. The trachea was covered with cotton thread. An incision was done carefully on the exposed trachea. After that, a catheter about 0.5 cm long was placed in the trachea. The trachea was wrapped in cotton thread to stabilise the catheter. Afterward, 1 ml of HBSS solution and 100 mM EDTA were added to a syringe. The salt and EDTA solution was then gently injected into the lungs using a syringe that was attached to a catheter. The fluid was gradually aspirated from the solution by rubbing the rat's thorax. The syringe was taken out, and the lavage fluid was collected in an EDTA tube that had been chilled. This procedure was repeated for 2-3times.

Biochemical estimation

1. Lung weight

After sacrificing animal Lung was dissected and washed in normal saline and weighed on the weighing balance. Weight of Lung was compared with each group. Weight variation was recorded.

2. Blood cells and Serum Biomarkers

Blood Eosinophil count: Blood collected on 29th day in EDTA tubes were sent for blood eosinophil count to Harsh Laboratory, Vadodara

Blood Neutrophil count: Blood collected on 29th day in EDTA tubes were sent for blood neutrophil count to Harsh Laboratory, Vadodara

Total WBC count of Blood: Blood collected on 29th day in EDTA tubes were sent for total WBC count to Harsh Laboratory, Vadodara

Serum IgE: Blood collected om 29th day in Citrate tubes were sent for serum IgE testing to Harsh Laboratory, Vadodara.

Serum Albumin: This was estimated by True Chemie Albumin Test Kit.

3. BALF Cell Count

Eosinophil count in BALF: BALF fluid collected on 29th day in EDTA tubes were sent for BALF eosinophil count to Parul Sevashram Hospital, Vadodara.

Neutrophil count in BALF: BALF fluid collected on 29th day in EDTA tubes were sent for BALF neutrophil count to Parul Sevashram Hospital, Vadodara.

BALF Albumin: This was estimated by True Chemie Albumin Test Kit.

Histopathological evaluation

Rats were slaughtered at the conclusion of the therapy, and the lung tissue was stored in 5% formalin while being blotted clean of blood and tissue fluids. The tissues were completely cleaned with 70% alcohol after 24 hours, followed by dehydration in escalating alcohol concentrations (70-100%). Tissues were first dehydrated in pure

alcohol, then treated with toluene, xylene (50:50), paraffin wax in toluene, and finally in 100% wax (paraffin wax, 60–62°C), after which the tissue was embedded in wax. Serial sections between 5 and 15 m thick were cut on a Leitz microtome in a horizontal plane and mounted on a glass slide using egg albumin in a solution of 50% v/v glycerine. They were then stained for three to five minutes with 10% haematoxylin, and the staining was made more intense by submerging them in flowing water. The haematoxylin-stained slices were treated with xylene, mounted in DPX, and then stained with 10% eosin for 2 minutes. They were then immediately passed through progressively stronger alcohols. With the use of a Magnus Olympus cx23 photomicroscope, the slices were examined, and the necessary locations were photographed. Magnifications of 40X and 100X were used to see the portions.

Statistical analysis

The mean ± S.E.M. is used to express all values. One-way ANOVA and the Sidak's multiple comparisons test, when necessary, were used to examine the statistical significance between more than two groups using a computer-based fitting programme (Prism, GraphPad 9.5.0). When $p<0.05$, differences were deemed statistically significant.

RESULTS

Phytochemical investigation

Methanolic extract of *Boswellia serate* and Water extract of *Mangifera indica leaves*were exposed to phytochemical assessment for checking the occurrence of numerous chemical constituents **(Table 1)**.

Evaluation parameters of suspension

Polyherbal suspension was subjected to evaluation for various parameters such as Redispersibility, Flow rate, Particle size, pH, Sedimentation volume, Viscosity **(Table 2)**.

Acute oral toxicity study

OECD-423 (Acute Oral Toxicity-Acute Toxic Class Method) was performed on Sprague Dawley Rats.

Initial Study

Acute toxicity testing was done at a dosage of 2000 mg/kg. skin, fur, eyes, and mucous membranes did not show any changes. Additionally, the respiratory, autonomic, central, and somatomotor nerve systems as well as the behavioral pattern were all normal. Tremors, convulsions, salivation, diarrhoea, lethargy, and coma were not seen **(Figure 1)**.

Confirmatory Study

Acute toxicity i.e., Confirmatory study was done at dosage of 2000mg/kg. skin, fur, eyes, and mucous membranes did not show any changes. Additionally, the respiratory, autonomic, central, and somatomotor nerve systems as well as the behavioral pattern were all normal. Tremors, convulsions, salivation, diarrhoea, lethargy, and coma

were not seen **(Figure 2)**.

Evaluation of polyherbal formulation

A. Weight of Lung

In **Figure 3**, weight of Lung of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group, Standard Control group showed slight decrease in Weight of lung, similarly Test Low group (T-1) showed significant decrease, Test High group (T-2) showed slight decrease in Weight of lung in comparison to Disease Control group.

2. Blood cells and serum biomarkers

Blood Eosinophil count

In **Figure 4** Eosinophil Count % of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group Standard Control group showed slight decrease in Eosinophil Count % similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Eosinophil Count % in compared to Disease Control group

Blood Neutrophil count

In **Figure 5** Neutrophil Count % of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group, Standard Control group showed slight decrease in Neutrophil Count %, similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Eosinophil Count % in compared to Disease Control group.

Total WBC Count

In **Figure 6** Total WBC Count×10^3/mm^3 of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group, Standard Control group showed slight decrease in Total WBC Count×10^3/mm^3 similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Total WBC Count×10^3/mm^3 in compared to Disease Control group.

Serum IgE

From the above mentioned data Serum IgE of Disease Control group was increased in comparison to N.C group. While, in comparison to D.C group, S.C groups and T1 and T2 groups showed decrease in Serum IgE **(Table 3)**.

Serum Albumin

In **Figure 7** Serum Albumin of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group Standard Control group showed slight decrease in Serum Albumin similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Serum Albumin in compared to Disease Control group.

3. BALF Cell Count

BALF Eosinophil count

In **Figure 8** Eosinophil Count BALF % of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group, Standard Control group showed slight decrease in Eosinophil Count BALF %, similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Eosinophil Count BALF % in compared to Disease Control group.

BALF Neutrophil count

In **Figure 9** Neutrophil Count BALF % of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group, Standard Control group showed slight decrease in Neutrophil Count BALF %, similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in Neutrophil Count BALF % in compared to Disease Control group.

BALF Albumin

In **Figure 10** BALF Albumin of Disease Control group was significantly increased in compared to Normal Control group. While, in comparison to Disease Control group Standard Control group showed slight decrease in BALF Albumin similarly Test Low group (T1) showed significant decrease, Test High group (T2) showed slight decrease in BALF Albumin in compared to Disease Control group.

Histopathological investigation

From the Histopathological Images of Lungs of the groups done, it is being observed that Test group low dose(T1) polyherbal formulation has shown decrease in the amount inflammatory cells and there is less swelling in lung cells in comparison to the Standard control group (SC), Test - high dose group(T2) and Disease control Group (DC) **(Figure 11)**.

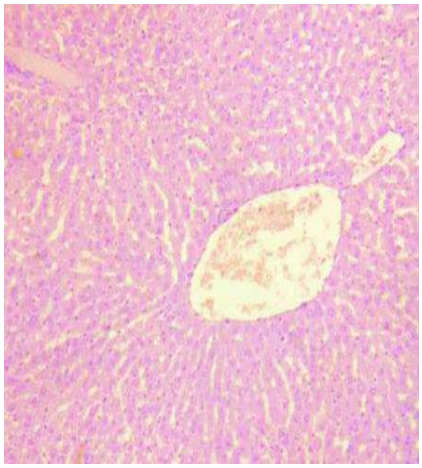
Table 1: Identification Test for Major Constituents

Types of Phytochemical Constituents	Methanolic extract of *Boswellia serrate*	Water extract of *Mangifera indica* leaves
Alkaloid	+ve	+ve
Flavonoid	+ve	+ve
Carbohydrates	+ve	+ve
Cardiac Glycosides	-ve	+ve
Starch	-ve	-ve
Tannin	-ve	+ve
Saponin Glycosides	-ve	+ve
Terpenoid	+ve	+ve
Anthraquinone	-ve	-ve
Steroids	+ve	+ve
Volatile oil	+ve	-ve
Coumarin	-ve	-ve
Resin	+ve	+ve
Amino Acid	-ve	-ve
Protein	-ve	-ve
Phenol	-ve	-ve

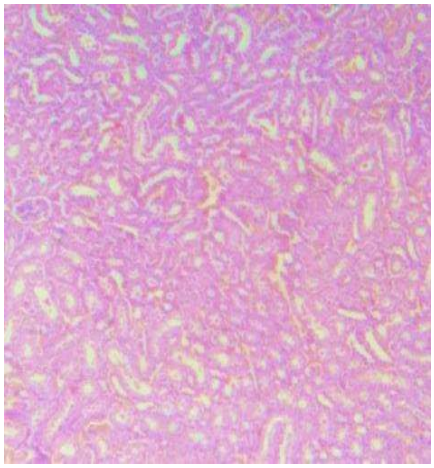
(+ve) Indicates positive result (-ve) Indicates negative result

Table 2: Evaluation Parameters of Suspension

Parameters	Result
Redispersibility	Good
Flow rate	0.83 ml/sec
Particle size	18 µm
pH	5
Sedimentation volume	2 ml in 100 ml of suspension
Viscosity	234 mPa.s

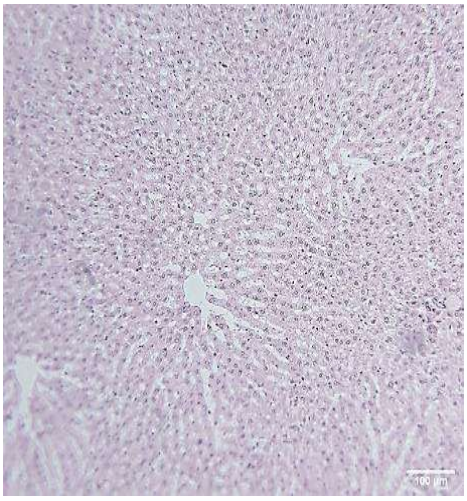


A. Liver
Normal histological structure of the central vein & demonstrating normal looking hepatic lobule & hepatocytes surrounding a central vein.

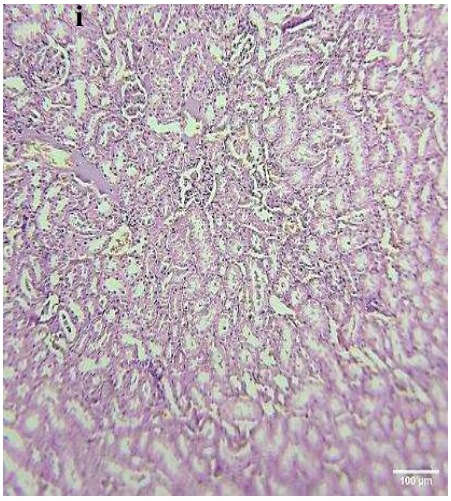


B. Kidney
Normal histological structure of the of glomeruli, tubules were seen.

Figure 1: Histological Investigation of liver & kidney (Initial Study)



A. Liver
Normal histological structure of the central vein & demonstrating normal looking hepatic lobule & hepatocytes surrounding a central vein.



B. Kidney
Normal histological structure of the of glomeruli, tubules were seen.

Figure 2: Histological Investigation of liver & kidney (Confirmatory Study)

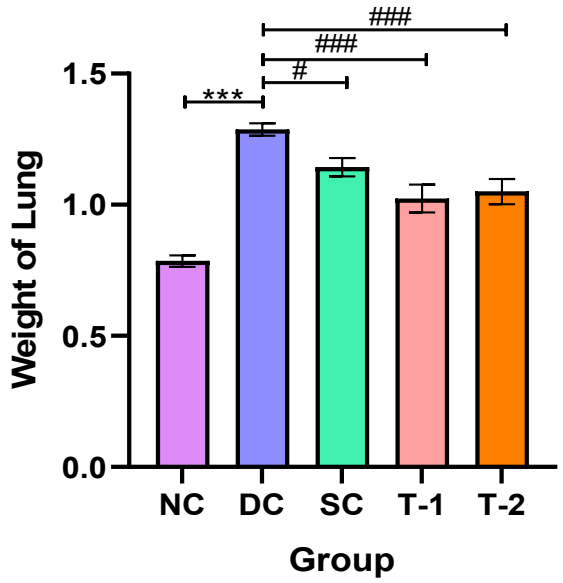


Figure 3: Effect of PHF on Lung Weight of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while #(P<0.05), ###(P<0.001) compared to Disease Control**

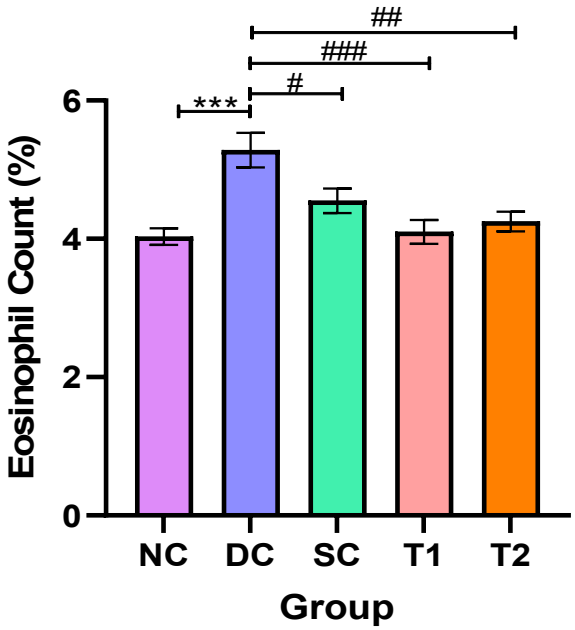


Figure 4: Effect of PHF on Blood eosinophil count of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while #(P<0.05), ## (P<0.01), ###(P<0.001) compared to Disease Control**

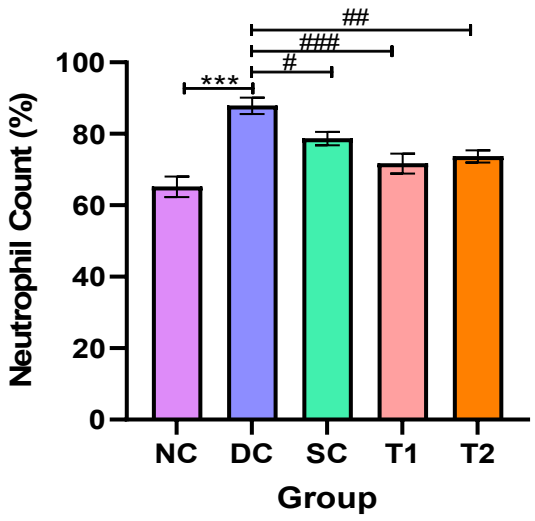


Figure 5: Effect of PHF on Blood neutrophil count of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while #(P<0.05), ## (P<0.01), ###(P<0.001) compared to Disease Control**

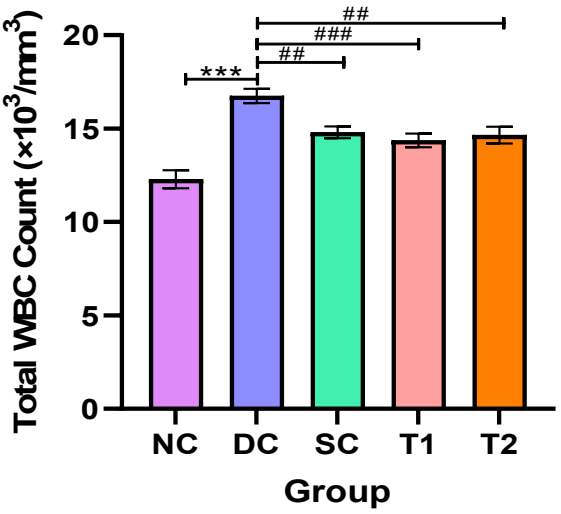


Figure 6: Effect of PHF on Total WBC count of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while ## (P<0.01), ###(P<0.001) compared to Disease Control**

Table 3: Effect of PHF on SerumIgE of Sprague Dawley Rats

GROUP	TREATMENT	SERUM IgE
I	NC	>1.2 IU/ml
II	DC	< 1.2 IU/ml
III	SC	>1.2 IU/ml
IV	T-1	>1.2 IU/ml
V	T-2	>1.2 IU/ml

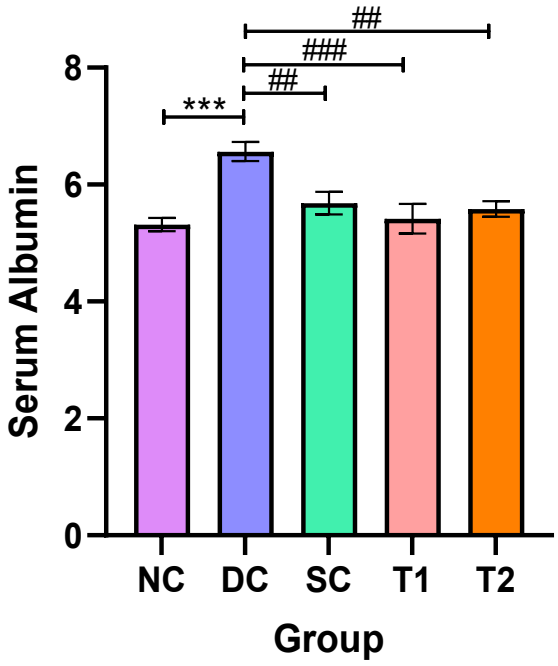


Figure 7: Effect of PHF on Serum Albumin of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while ## (P<0.01), ###(P<0.001) compared to Disease Control**

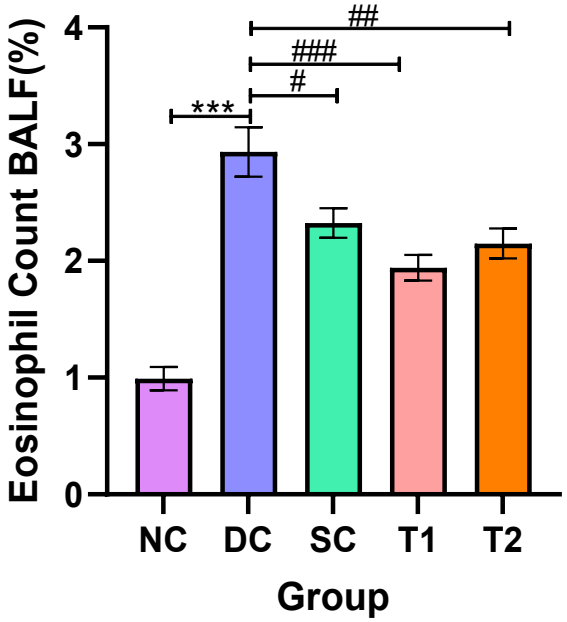


Figure 8: Effect of PHF on BALF Eosinophil count of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while # (P<0.05), ## (P<0.01), ###(P<0.001) compared to Disease Control**

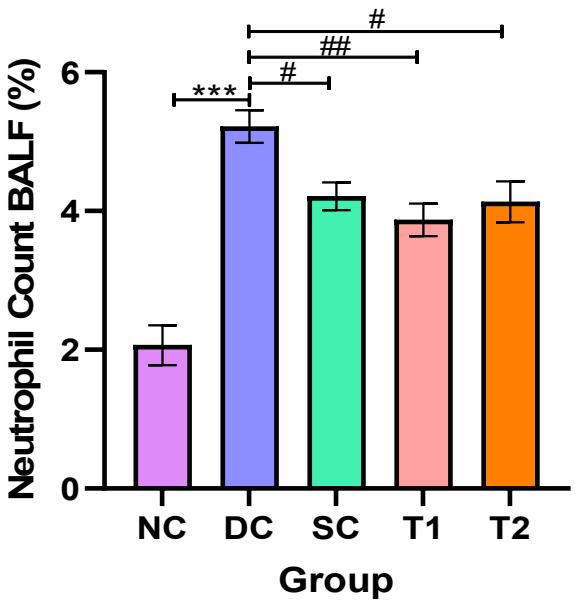


Figure 9: Effect of PHF on BALF Neutrophil count of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while # (P<0.05), ## (P<0.01) compared to Disease Control**

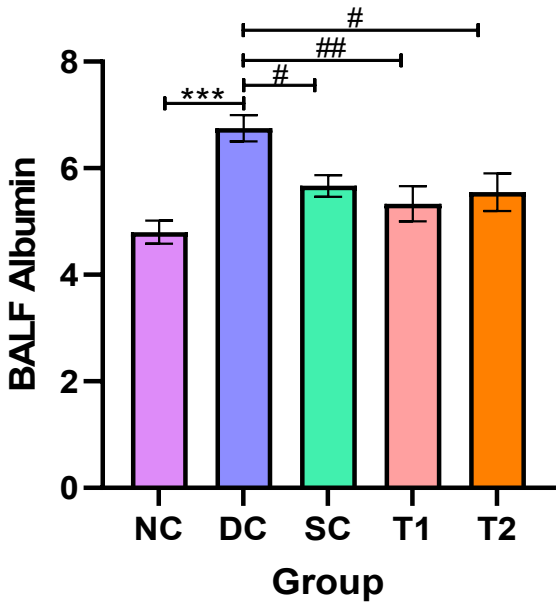


Figure 10: Effect of PHF on BALF Albumin of Sprague Dawley Rats in Disease control (DC) *(P<0.001) compared to Normal control (NC), while # (P<0.05), ## (P<0.01) compared to Disease Control**

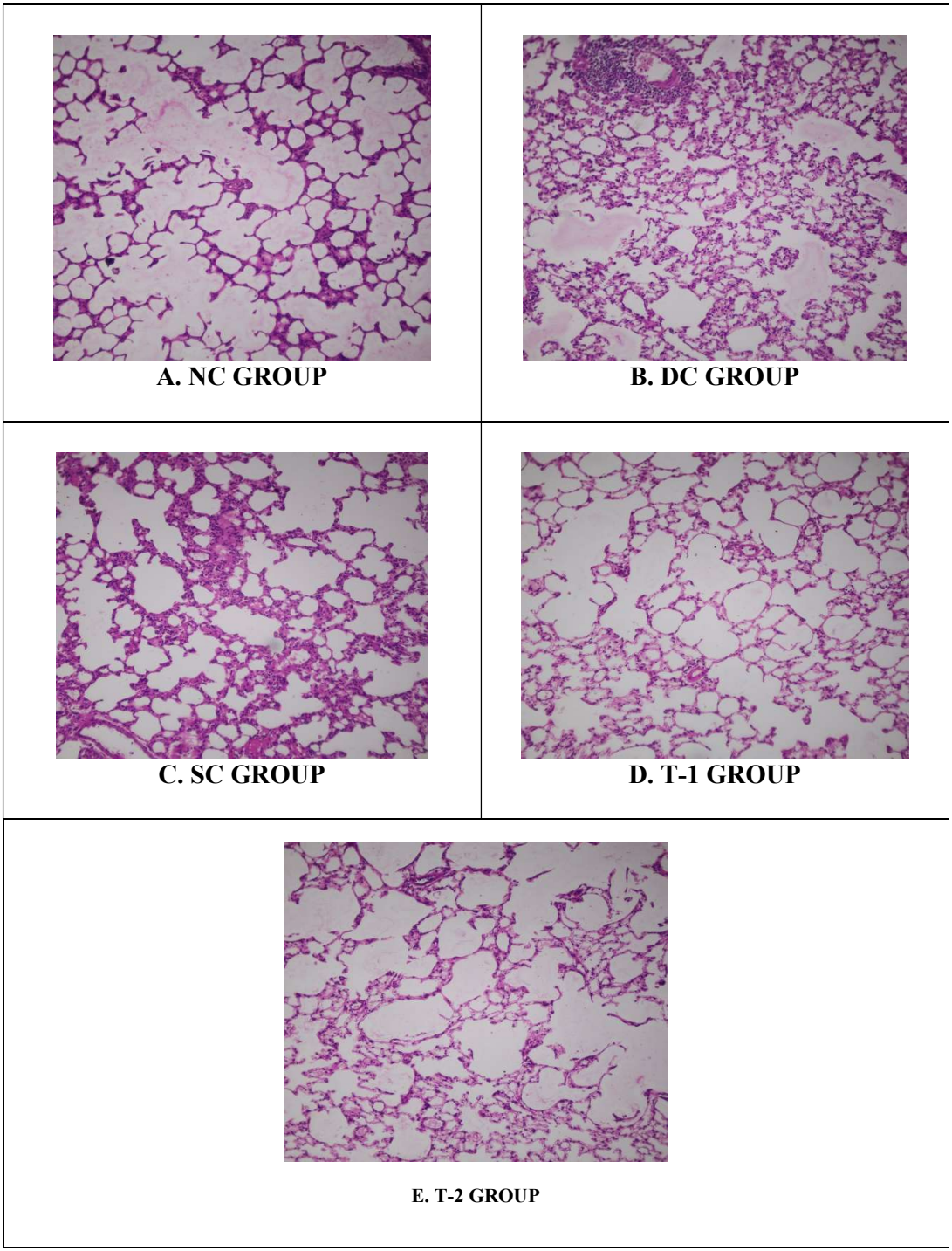


Figure 11: Histopathology

DISCUSSION

When tracheobronchial smooth muscle is oversensitive to various stimuli, it narrows the airways and is frequently accompanied by increased secretion, mucosal edema, and mucus clogging **[4]**. There are three possible outcomes during an asthma attack are bronchospasm, inflammation and mucus production **[10]**. Asthma was rated 34th among the top causes of disease burden in

2019 according to GBD, accounting for a fifth of all disability adjusted life years (DALYs) from chronic respiratory disorders. Asthma was predicted to be responsible for 21.6 million DALYs across all age groups worldwide. The 10.2 million YLD were responsible for over half of the asthma burden **[9]**. The development of airway remodelling appears to be heavily influenced by chronic persistent inflammation and the release of several cytokines, chemokines, and growth factors from inflammatory and epithelial cells[13] . The animals of group (II – V) were given i.p and s.c 0.66ml of allergen suspension containing ova –albumin (1 mg) and Al(OH)3 (300mg) in saline, then challenge i.n. with 1% ova for the induction of Asthma. Before initializing of treatment biochemical estimation was performed and was compared to Normal control Group (Group-I). Normal control Group (Group-I) was considered as base line for biochemical analysis **[10]**. Allergic asthma models have historically been induced using ovaalbumin (OVA). The mice create anti-OVA IgE antibodies during this sensitization period, and these antibodies attach to IgE receptors on mast cells. After this sensitization, the animal is intratracheally challenged with OVA, resulting in OVA cross-linked IgE on mast cells and degranulating mast cells as a result. Then the animal displays asthmatic symptoms **[22]**. Montelukast Sodium is a Leukotriene antagonist. It functions by preventing leukotriene D4 from acting in the lungs, which reduces inflammation and relaxes smooth muscle. It has various side effects like viral infection, abdominal pain, eczema etc. Polyherbal formulation (PHF) consisted of *Boswellia serrate* extract, *Mangifera indica* leaves extract. These all were combined on the basis of effects of the phytoconstituent basically Boswellic acid and Mangiferin in herbs on different pathophysiological steps of Asthma. Boswellic acid potently present in Boswellia serrate acts by three different mechanism that is by inhibiting 5-LOX, by inhibiting C2 Convertase and by inhibiting Leukocyte Elastase **[24-25]**. Mangiferin potently present in Mangifera indica leaf extract acts on Th1/Th2 cytokines by following four different mechanism that is by reducing eosinophil and total inflammatory cell infiltration, lowering PGD2 in BALF and ovalbumin specific IgE in serum, reducing levels of Th2 related cytokines like IL-3, 4,5,9,13,17, Tumor Necrosis Factor-α and elevated blood levels of Th1 related cytokines (INF, IL-2, 10,12) **[23]**. This polyherbal formulation was given to Test Group with Low Dose and Test Group with high dose which is denoted by T1 and T2 respectively. Low and high dose was selected on the basis of Acute oral toxicity-OECD 423 guideline of the polyherbal formulation. Polyherbal formulation was

administered orally for 28 days to T1 and T2. After 28 days all the biochemical parameters, Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung was done. After 28 days i.e at the conclusion of study it is evident that Group III (DC) that is Disease Control showed significant increase in Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung. Histopathological interpretation shows severe inflammation and in alveolar duct is reduced due to inflammation. On contrary, in Group –IV (T2) that is Low dose of polyherbal formulation shows significant decrease in Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung. Histopathological interpretation shows observable reduction in inflammation and in alveolar duct was reduced due to inflammation. In Group-V (T2) that is high dose of polyherbal formulation shows moderate decrease in Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung. Histopathological interpretation shows severe inflammation and in alveolar duct is reduced due to inflammation. In Group-II (SC) that is Standard control shows mild decrease in all the Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin; weight of lung. Histopathological interpretation mild decrease in swelling of alveoli, and increase in inflammatory cells in comparison to DC group. While Group-I (NC) that is Normal control group possess normal level of all these biochemical parameters and also normal Histopathological interpretation. Results of research showed that this Polyherbal formulation (PHF) works in Asthma induced by Ova-albumin in rats by above mechanisms, inhibiting 5-LOX, by inhibiting C2 Convertase, inhibiting Leukocyte Elastase, acting on Th1/Th2 cytokines by following four different mechanism that is by reducing eosinophil and total inflammatory cell infiltration, lowering PGD2 in BALF and ovalbumin specific IgE in serum, reducing levels of Th2 related cytokines like IL-3, 4,5,9,13,17, TNF-α and elevated blood levels of Th1 related cytokines (INF, IL-2, 10,12). Hence, this Polyherbal formulation has promising effect to protect as well to cure Asthma.

CONCLUSION

The utmost precedence prolonged disease in children with a high morbidity and low mortality rate is asthma, a serious non-communicable disease (NCD) that affects both children and adults. A research from

the World Health Organization (WHO) from 2021 claims that 15 to 20 million people in India, including people of all ages, suffer from asthma. Asthma is characterised by, inflammation of the airways, bronchial hyper-reactivity andreversible airways obstruction. There are various types of treatment and management options but relief offered by them is symptomatic and they show adverse effects. The animals of group (II – V) were given i.p and s.c 0.66ml of allergen suspension containing ova – albumin (1 mg) and Al(OH)3 (300mg) in saline, then challenge i.n. with 1% ova for the induction of Asthma on 0, 8 and 28 day through i.p, s.c and i.n respectively. This showed significant increase in Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung and causes increase severe inflammation and in alveolar duct is reduced due to inflammation. Polyherbal formulation (PHF) normalize the level of in Blood Eosinophil, Neutrophil, Total WBC count, Serum IgE, Serum Albumin, BALF Eosinophil, Neutrophil, BALF Albumin and weight of lung and also decreases inflammation and in alveolar duct is normalized. All the observation shows that Polyherbal formulation can be used to prevent asthma in rats induced by ova-albumin. This Polyherbal formulation in addition has the ability to prevent asthma in much significant way than the standard drug, Montelukast sodium. This calls for additional research examining the significance of the PHF extract's pharmacologic potential in treating asthma by identifying the molecular pathways of action as well as cell line studies.

Abbreviations

WHO- World Health Organization, NCD- Non-Communicable Disease, GBD- Global Burden of Disease, DALYs- Disability Adjusted Life Years, LABA- Long Acting Beta-2 Agonist, IL- Interleukin, TNF- Tumour Necrosis Factor, CCR3- Chemokine Receptor 3, PGD2- Prostaglandin-2, TSLP- Thymic stromal Lymphopoietin, ILC- Innate lymphoid cells, Th- T-helper , CMC- Carboxy methyl cellulose, 5-LOX – 5-Lipoxygenases, BALF- Bronchoalveolar lavage fluid, PHF- Polyherbal Formulation.

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