'Animal and clinical study for Treatment of Asthma using Intranasal inhalation of Himalayan salt'

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ii

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Table of Contents

List of Figure

List of Tables

Abbreviations:

ALA- American Lungs Association

COPD- Chronic obstructive pulmonary disease

BHR- Bronchial hyper-responsiveness

ICS- inhaled corticosteroids

SABAs- short-acting beta-agonists

VC- vital capacity

PEF- peak expiratory flow

CBC- Complete blood count

OVA- ovalbumin

PBS- Phosphate buffer saline

FDA- Food and Drug Administration

AHR- airway hyper-responsiveness

HE- Hematoxylin and Eosin

Abstract

Asthma is a non-communicable disease (NCD) that affects airways by narrowing their diameter and eventually causing inflammation in lungs. This has become one of the most frequently diagnosed disorders in the past few decades. In addition, more death due to asthma is recorded in low income countries where treatment is a challenge due to related cost. The purpose of this study is to investigate effects of salt therapy in asthma disease based on clinical study by using human specimen and vet lab study by using Wister rats as asthma model. A cross-sectional survey-based and experimental study was conducted on asthma patients at the Khewra salt mines sanatorium. The survey-based study comprised 107 patients of which 8 were randomly selected for experimental study. Patients of both genders from ages 2 months to 55 years were part of this study. Participants were asked to stay in the Khewra salt mines sanatorium for 10 hours for 10 days. CBC test was performed before and after salt therapy to check the role of pulmonary immune cells in asthmatic patients for diagnostic purposes. Statistical analysis was made based on immune cell count including platelets, eosinophils, monocytes, neutrophils, and lymphocytes. Moreover, in vet lab based study, rats are extensively used as asthma model globally, for this study 5 treatment groups of Wister rats were used. Asthma was induced in rats by combination of Ovalbumin (OVA), alum and Phosphate Buffer Saline (PBS) solution and then diseased rats were exposed to intranasal inhalation of salt aerosols of different concentrations (0.5%, 1%, 3%, 5% and 6%) of pink salt solution for treatment purpose. For obtaining findings, blood samples from their hearts were collected for complete Blood Count (CBC) test for immune cells analysis. The rats were later dissected for histological analysis of lungs. The results of survey-based study indicate that salt therapy is emerged as an advanced treatment method for asthma patients as it reduces frequency of asthma attacks and minimizes use of medication. It was also observed that after salt therapy count of immune cells decreased in all 8 patients. The results of paired sample t-test gave a p-value of less than 0.05 in the case of all 5 immune cell counts, which shows salt therapy is an effective treatment for asthma patients. Vet lab study results show that body weight significantly increased from the start of treatment phase to the end of experiment in all treatment groups. However, lung weight analysis revealed remarkable decrease in diseased rats. CBC tests show a significant decrease in immune cells count of treatment rat groups with different aerosols concentrations. The histological results of different treatment groups were then compared with normal and diseased rats. It is observed that salt treatment proves to be significantly effective way for treating asthma through intranasal salt aerosols inhalation. This study shows that salt therapy can be used as a treatment for asthma.

Keywords: Asthma; Chronic respiratory disorder; Animal study; Histological Analysis and CBC test; Pink salt solution; OVA and alum

Chapter 1

1. INTRODUCTION

1.1. Asthma

Asthma is a persistent disease that damages airways. It gives rise to wheezing and can make it difficult to breathe. Some provokes include direct contact to an allergen or irritant, viruses, emotional stress and exercise. During an asthma attack, muscles around airways become tighten, walls of airways swelled up, and transport of air in and out of lungs become difficult. The percentage of asthma has been expanding since 1950s and is approximated as 300 million individuals worldwide. It is now one of the most persistent diagnosed diseases in the globe and looks to be more in urban population. The occurrence of asthma varies between countries, with percentage rate of 15.3% in England, 14.1% in Canada, 10.9% in the USA, 6.9% in Germany, 6.7% in Japan and 4.5% in Italy[1]. In 2018, over 1.7 million (8.1%) teenagers 20–24 years were diagnosed asthma disease[2]. Additionally, the asthma occurrence was greater among women than men. Many studies indicated that the atopy is greatly linked with asthma, mostly in young adults, teenagers and children which may show an increase in asthma percentage in coming years. Therefore, to control the asthma rate globally different treatment methods are used included drug-free treatment[1].

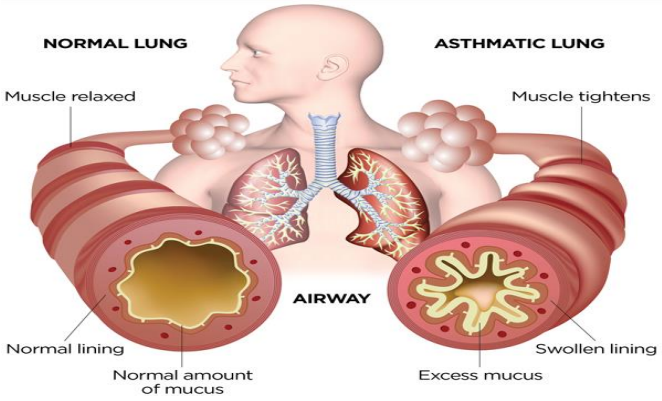


Figure 1. Comparison between airways of normal and asthmatics patients (https://community.aafa.org/)

1.2. Types of Asthma

Although there are many distinct methods and reasons why asthma might spread, the triggers are always the same. They may involve a wide range of categories, including:

1.2.1. Childhood asthma

The most common illness among children is asthma. Although it can manifest at any age, children are more likely to experience it than adults. The American Lung Association (ALA) lists some typical

causes of childhood asthma as: respiratory illnesses and colds; allergens contaminants in the air, both indoors and outdoors; exposure to cold air; a sudden shift in temperature; excitement; worry; exercise. If a kid develops asthma, seeking medical assistance is imperative since the condition can be life-threatening. A doctor can suggest some effective strategies for treating the illness. Additionally, in certain cases, as the child ages, their asthma may get better.

1.2.2. Adult-onset asthma

At any age, including as an adult, asthma can develop. Asthma risk factors for adults include some of the following: respiratory conditions; allergies and exposure to allergens; hormonal influences, Obesity, stress, and tobacco smoke.

1.2.3. Occupational asthma

The development of occupational asthma is brought on by exposure to an allergen or irritant present at work. Additionally, roughly 21% of working adults have discovered that their symptoms worsen while they are at work. because a person may be exposed to asthma triggers in both indoor and outdoor work environments.

1.2.4. Eosinophilic asthma

It is the kind of asthma when standard treatments are ineffective. While some individuals with eosinophilic asthma take conventional asthma drugs, others may utilize particular biologic therapy. One form of biologic drug reduces eosinophil counts because higher eosinophil counts are associated with allergic reactions that might exacerbate asthma symptoms.

1.2.5. Seasonal asthma

When individuals are exposed to allergens that are present in their immediate environment during specific periods of the year, this type of asthma develops. For instance, pollen in the summer or chilly air in the winter may exacerbate seasonal asthma symptoms.

1.2.6. Genetic factors

The ALA asserts that a person's lifelong risk of developing asthma may be influenced by genetics. A person is more likely than others to have asthma symptoms if one or both of their parents have the disease.

1.3. Asthma diagnostic tests

To assess how well the lungs are functioning, the doctor could do a lung function test. Spirometry testing is the procedure that medical practitioners utilize most frequently to examine lung function and diagnose asthma. A person taking the test will need to take a deep breath in and then violently exhale into a tube. The tube attaches to a device called a spirometer, which measures how quickly patients exhale air from their lungs.

Other diagnostic tests include:

• The challenge test: Using this test, a clinician can assess how breathing patterns are affected by stimuli like cold air, exercise, or inhaled drugs.

• Skin or blood tests may be used by a doctor to test for allergies.

• Blood test: A doctor may advise a blood test to look for elevated levels of eosinophil and immunoglobulin E, an antibody that patients with allergic asthma's immune systems make.

1.4. Treatment

Asthma treatment choices are expanding and getting better. The goal of treatment is to: improve a person's ability to breathe; lessen the frequency of attacks; and increase the amount of activities they can engage in. In recent years, asthmatics have had access to both long-term control drugs and medications for immediate relief. When taken regularly, long-term control drugs lower the frequency of episodes while helping to lessen symptoms quickly.

Asthma medications recently contain:

- muscles around the airways are become relaxed by using long- and short-term bronchodilators

- antibacterial medicine for a bacterial pneumonia or bronchitis
- long-term maintenance by using anti-inflammatory medication such as inhaled corticosteroids, or for an acute attack oral steroid
- combining corticosteroids and bronchodilators[3].

1.5. Drug-Free Treatment Method

Since the beginning of time salt has been existing all around world and utilized in different ways from ancient times: as a preservative, on snowy and iced roads, as an ordinary cooking ingredient and also as currency in past. Salt is one of the crucial constituents that establish life. Salt has also been used in several healing, medicinal and therapeutic ways involving nebulizers, saline solution, skin scrubs and salt baths etc. mainly called as wet salt therapy. In our present society, due to efforts of technological revolutions, another form of salt therapy has appeared where the major health advantage expected from the salt's quality to absorb humidity. It is known as Dry Salt Therapy and is also called as speleotherapy. In present day, salt therapy is being given around Europe, Australia, UK, Canada and other places as a natural substitute and complimentary way of enhancing extensive fitness, respiratory problems, dermis conditions, detoxification, athletic performance and more. There are particular businesses and facilities found throughout the world that are exclusively concentrated on giving dry salt therapy. They are generally assigned in salt caves, salt rooms and other names. There are variety of businesses starting dry salt therapy such as Day Spas, fitness Centers, Yoga Studios, Medical Spas, wellness Clubs and fitness businesses and professionals[4].

1.5.1. History and Background

Salt therapy started in Eastern Europe's salt caves and mines. Workers extracted salt by chiseling, crushing, and pounding, which discharged micro-sized salt particles into the air. Underground conditions such as air pressure, circulation, humidity, and temperature all played a role in the high-quality habitat. Miners who inhaled these salt particles reaped a variety of natural health benefits. Surprisingly, despite the inherent dangers of mining, salt miners were in superb health, with few respiratory problems and youthful skin. In Poland's Wieliczka Salt Mine, Dr. Feliks Boczkowski, a capable physician and astute businessman, founded the first health resort complex in 1839. He provided salt baths using natural brine from underground and noted that remaining underground may be more effective than inhalations for asthma treatment. Dr. K.H. Spannahel, a German physician, discovered the respiratory health benefits of persons finding sanctuary in salt mines and caverns during WWII in 1949. He proposed the Klyutert cave as a methodical technique to examining climatological variables and demonstrating the medical efficacy of underground settings. These research, together with the work of Hungarian Geologist Dr. H.

Kessler, helped to lay the framework for current Speleotherapy, which takes advantage of the therapeutic benefits of underground habitats. Professor Mieczyslaw Skulimowski became the official physician of the "Wieliczka" Salt Mine in 1958 and began treating patients in salt chambers, paving the way for Subterraneotherapy, also known as the Skulimowski method, which concentrates on underground "salted" habitats. The subterranean "Kinga" Allergy Treatment Spa, the first of its kind, was created in the "Wieliczka" Salt Mine in Poland in 1964. Professor Skulimowski became its inaugural Director, devoting his time to aiding people with respiratory issues and researching overall health advantages. His effective tactics expanded to nearby salt mines and caves in Europe and former Soviet Union territories. The first speleo-hospital was established in the Solotvyno salt mine in Ukraine in 1968. As the medical community's interest expanded, so did the need for more convenient access to perform particular tests and research. The first Halotherapy device was developed in 1985 by the Institute of Balneology in Odessa, Russia, in collaboration with the salt caves of Uzhgorod. To spread salt particles into the air, this contraption reproduced the grinding and crushing of salt. Thus, halotherapy evolved in the medical milieu of the former Soviet Union. Following the demise of the Soviet Union in 1991, knowledge about halotechnology and established Halotherapy protocols were widely available around the world. Beyond the USSR, halotherapy quickly found a home in commercial and wellness contexts, expanding to Eastern and Western Europe, Canada, Australia, and, finally, the United States. [5]

Figure 2. Salt mines of Wieliczka (Recommended from information)

1.5.2. Salt therapy

Salt therapy consists of mainly two types, wet salt therapy and dry salt therapy. Wet salt therapy involves salt bath, saline solution, salt scrub and nebulizers. Wet salt therapy is utilized in gargling solutions, sole drinking water and flotation tanks. Dry salt therapy involves use of dry salt in an environment that is clear of humidity and moisture. Dry salt therapy takes place under controlled conditions such as in the form of salt room, salt chamber, salt caves etc. Here there are some expressions used to explain dry salt therapy such as Speleotherapy and Halotherapy. The salt therapy association

describes these terms as

- Speleotherapy is a therapy that is given beneath the Earth's surface using the climate conditions and in natural caves salt air is found naturally.
- Halotherapy is a treatment that is administered in a salt therapy room using a specialized equipment called a halogenerator to disperse a precise size dry salt aerosol.
- Two different types of salt rooms—active salt rooms and passive salt rooms—offer dry salt therapy in a synthetic environment.

1.5.2.1. Active salt room

Active salt rooms have a halogenerator and are essentially depends on giving a precise salt aerosol in the air to be inspired and revealed to the skin. This can be attained only by a particular piece of instrument called halogenerator. 99.9% pure sodium chloride is put down into the halogenerator, which then squashes and grinds the salt into micro-sized particles and spreads the salt aerosol into the air. Dry salt therapy of this type is called Halotherapy.

Figure 3. Layout of Halotherapy room with halogenerator (https://breathesaltandsauna.com/)

1.5.2.2. Passive salt room

Passive salt rooms are also artificially constructed spaces that are filled with a variety of salts, such as Himalayan salt, Dead Sea salt, Mediterranean salt, rock salt, etc., but they lack halogenerators. Generally, passive salt rooms are created to check the climate by controlling the airflow, temperature and humidity to permit an environment free of pollutant and allergens. Actually, these passive salt rooms are trying to cope Speleotherapy, depends on the natural salt caves presence of natural salt cave all over Europe, though it is in an artificial environment. Speleotherapy happens in natural salt cave environments below ground with particular conditions that involves air temperature, atmospheric pressure, composition of air, humidity and other components that cannot be replicated in an artificial

environment. Devoid of halotherapy equipment, it is not accepted as halotherapy. Generally, it is a passive salt room that gives a great place for meditation, relaxation and enhances psycho-emotional health. Although there is more research and clinical studies on both Speleotherapy and Halotherapy, main benefits of dry salt therapy depend on the inspiration of specific sized salt particles into the respiratory system and also skin being exhibited to these micro-size salt aerosols. This is only achievable in an artificial environments by using an equipment such as a halogenerator[6].

1.6. Working principal of Himalayan salt

There are three primary characteristics of pure sodium chloride, NaCl, known as salt:

1. Salt is super absorbent (especially when it is completely dry)
2. Salt is anti-bacterial
3. Salt is anti-inflammatory

Dry salt has the capacity to attract and absorb allergies, poisons, and other foreign things in the lungs and respiratory tract when inhaled. This procedure can aid in the reduction of inflammation and the opening of airway passageways. Pure sodium chloride (NaCl) particles have a particular crystal structure that serves to eradicate hazardous chemicals. This fundamental mechanism underpins how dry salt treatment can help people with respiratory disorders like allergies, asthma, bronchitis, cystic fibrosis, COPD, and others. Furthermore, dried salt has qualities that absorb pollutants and have antibacterial effects, which can be beneficial for skin diseases such as acne, psoriasis, and eczema. It also controls the skin's microcirculation. The salt particles have the potential to penetrate beneath the skin's surface, attracting moisture. As a result, cell regeneration is sped up, and skin suppleness improves. As a result, maintaining particular temperature and humidity levels, as well as proper ventilation, is critical in a dry salt room to ensure the efficacy of salt therapy and to offer a clean-air environment for those seeking its advantages. [8]

1.7. Beneficial therapeutic effects of Himalayan salt

Himalayan salt takes out from Khewra salt mines located near to Himalayas in Punjab, Pakistan. Himalayan salt has more mineral content that’s why it is pretty pinkish tint in color. Himalayan pink salt has about 84 natural minerals and trace elements that are functionally very useful in human body. Therefore, it is also known as white gold in Himalayas. Pink salt is existing in different forms such as salt bricks, salt candle holder, salt tiles, oil diffuser and pink salt lamps. Himalayan salt lamp act as a diffuser in different ways. When Himalayan salt lamp heats up, it attracts the water molecules from surrounding and then ionizes. After ionization, releases negative ions in the surroundings. Negative ions grab the allergens and pollutants in the surrounding. These negatives have calming effect on our nerves.

In our bloodstream these negative ions increase the level of serotonin hormone that regulates our mood, feelings, and happiness. Natural Himalayan salt mine environment is used to make patients inhale negative ions for treatment of many respiratory and pulmonary diseases like asthma[7]**.** Speleotherapy, or dry salt therapy, benefits people of all ages, including adults, children, athletes, and even animals. It is a natural therapy procedure with no negative side effects. Speleotherapy is extremely beneficial for boosting general well-being through a variety of ways. It effectively removes pollutants from the respiratory system, resulting in enhanced physiological functioning and skin attractiveness. It also has a favourable effect on the immune system. Furthermore, salt therapy helps to increase lung capacity, reduce stress, and promote healthier sleep patterns.

1.7.1. Lungs and Respiratory Hygiene

It is critical to maintain healthy lungs and a clean respiratory system for overall well-being and lifespan. The average adult breathes 12-15 times per minute, while small toddlers breathe 20-30 times per minute. The lungs are responsible for delivering oxygen to our red blood cells as well as removing carbon dioxide from the body. Furthermore, the respiratory tract functions as a vital defence mechanism by filtering contaminants and controlling the pH levels of our blood via CO2 management. Unfortunately, we are exposed to a variety of pollutants, airborne infections, bacteria, allergies, and other irritants on a regular basis. In this context, salt treatment offers a natural way to cleanse and detoxify the lungs while also boosting lung capacity and oxygen intake, revitalising the entire body.

1.7.2. Salt therapy upgrades Respiratory System

When inhaled, dry salt has antibacterial, mucokinetic, and anti-inflammatory characteristics that can effectively reduce inflammation and open airway channels throughout the respiratory system. Dry salt behaves like a sponge, collecting foreign things in the respiratory tract due to its remarkable absorbency. It functions similarly to a toothbrush, cleaning the respiratory system by removing foreign materials that are frequently responsible for various respiratory illnesses.

1.7.3. Respiratory conditions might improve through Salt therapy

Speleotherapy can be used to treat a variety of respiratory conditions, including:

- Asthma
- Allergies
- The common cold
- Bronchitis
- COPD
- Cystic fibrosis
- Sinusitis

- Ear infections
- Cough caused by smoking

When it comes to respiratory issues, inhaling dry salt and attending regular sessions are important variables that lead to positive outcomes.

1.7.4. Dermatological effects of salt therapy

Scientific study has revealed that dry salt therapy improves the overall quality and look of the skin. Salt therapy has therapeutic and aesthetic effects on the integument system, which serves as the skin's protective covering. This therapy improves ion channel function in skin cells and promotes electrophysiological activity. The salt promotes reparative and regenerative processes in the dermis, improves skin suppleness, stimulates hair development, and improves hair health. Furthermore, dry salt therapy increases skin microcirculation and cellular membrane activity, improving their effectiveness. As a result, dry salt therapy may be beneficial to those suffering from a variety of skin disorders, such as:

- Psoriasis
- Itching
- Eczema
- Swelling and Inflammation
- Dermatitis
- Dry and Flaky Skin
- Acne
- Rashes
- Skin Aging

1.8. Contraindications

Salt therapy is considered as a safe and effective practice for preventive and restorative health care. Salt therapy is not preferable for people with infectious diseases, fever, open wounds, lung cancer, severe hypertension, mental disorders and active tuberculosis. Pregnant women should ask from their doctor before taking salt therapy [9].

1.9. Objectives

The objective of this research included:

- Effect of salt therapy on respiratory disorders in real life.

- Combined effect of salt therapy with drugs that how effective is the drug therapy along with salt therapy.
- Survey Kheer Salt Mine sanatorium to collect data
- Salt Therapy is a completely natural, non-invasive, non-medical, side effect free therapy.
- It provides the therapeutic effect with clearance of respiratory tract. It is effective to inhibit the bacterial growth and also prevent the stimulation of inflammatory substances that causes asthma attack in patients.
- It is more effective technique for acute respiratory disease but also for chronic patients with combination of drug therapy.

It is important for people to have awareness of the modern techniques of treatment. Pakistan is providing salt therapy treatment in salt mines present in Khewra as natural source. But there is no proper setup available under controlled conditions for treatment of respiratory diseases. Therefore, this research will be a great contribution as natural therapy for treatment of respiratory disorders.

Chapter 2

2. LITERATURE REVIEW

2.1. Asthma

Asthma is a widespread chronic respiratory disorder that results narrowing airways and production of more mucus causes breathing issues, coughing and wheezing[1]. It is a complex airway disease that affects globally both children and adults. As specialist have identified that severe and persistent asthma occurs from heterogeneous conditions. These findings have helped to the development and testing of new classes of asthma treatments that focus on specific immune pathways. Developing therapies now allow specialists to give personalized medication to patients with severe asthma [32].

2.2. Pathophysiology

Airway obstruction is the main feature of asthma, which is produced by a reduction in the airway's diameter. The decreasing airways diameter is resulted by chronic inflammation of the airway wall, and activation of immune cells such as eosinophil, neutrophils, lymphocytes, and monocytes. A complex relation between these immune cells and with neighboring cells such as platelets results to the development of asthma such as BHR. Moreover, in severe forms of asthma, airway obstruction does not always normalize by medication or inhaler. In such patients, addition of life long mucus plugs in smaller airways can be fixed airway obstruction or other mechanisms, including airway remodeling[31]. In another clinical study, rats with mild to severe respiratory disorders results sniffling, dyspnea, ruffled hair coat and weight lose but lung weight increases due to thickness of airway walls and more mucus in airways. Major changes observed in rats affected with respiratory disorders are characterized by change in immune system function as neutrophils, plasma cells and lymphocytes results chronic bronchitis[35].

2.3. Medication

Recently, medications used for asthma control reduces airway inflammation and assist to lessen asthma symptoms; among these, inhaled corticosteroids (ICS) are the mainly used in the treatment of asthma, whereas quick-relief (reliever) or rescue medicines immediately ease symptoms that may originate acutely. It includes short-acting beta-agonists (SABAs) rapidly decrease airway bronchoconstriction (resulting relaxation of airway smooth muscles)[33].

2.3.1. Side Effects

But these medications also have some side effects such as fungal infection, sore throat, decreased bone density in adults and cataracts. Other side effects included muscle weakness, high blood pressure, high blood sugar, aggression, depression and sleep disturbance[34].

2.4. Therapeutic effects of Salt therapy

Research by Lemko et al. investigated impact of speleotherapy with a duration of 9-12 months in asthma patients. For speleotherapy patients would be stay in salt mines for 2 to 12 hours each day and therapeutic effect observed in a period of 3-4weeks. Microclimatic conditions of salt cave with temperature of 6-15°C and high humidity of 80-100 % was considered more effective. After examination of patients with continuous asthma varying from mild to moderate intensity, first therapeutic effect was noticed in 55 % of patients after 2 years, and after the 2nd or 3rd year in 60 % and 74 % of patients respectively. Consequently, asthma attacks were not noticed during period of 2 years in 42 % of asthma patients. In another study he conducted research on dyspnea patients by using speleotherapy to control development of dyspnea attacks in patients with situation alarming in initiation of bronchial asthma. Consequently, speleotherapy for 3-5 years reduces prevalence of 1st asthma attack in 5-8 %[10].

Chervinskaya et al. estimated efficacy of halotherapy in 87 patients of bronchial asthma. The control group of 15 patients endured placebo. Halotherapy course included 10-20 daily procedures of 1 hour. The positive change as decrease of bronchial resistance was noticed. No change in control group parameters were noticed after halotherapy sessions. After halotherapy, the clinical condition of 85% of patients with mild to moderate BA, 75% of patients with severe BA, and 98% of patients with chronic bronchitis improved. The patients were investigated 6-12 months after 1st halotherapy course. There was no aggravation of disease from 3rd to 12th month. 60% of them were utilized no medication and looked for no medical advice. In another trial, patients spent 45 minutes in a halotherapy room breathing in salted air that was pushed to the tiniest bronchi as well as the sinuses and nasal cavities. The salt chambers featured low humidity air (40-60%) and a temperature range of 22-24°. There was no need of special clothing in salt room. But advantages of halotherapy are greater if patients are bare-legged. Consequently, 12 to 20 sessions for 45 min are proposed for patients with chronic pulmonary diseases such as asthma, bronchitis, sinusitis, COPD[11].

Chernenkov et al. was used halotherapy in 29 patients from recovery of asthma attacks. The room was ventilated and temperature range 22-24^{o}C. The therapy session lasted after 45min resulted in enhancement of lung function by increased volume flow rate. In consequence, recovery from asthma attacks were needed 8-12 weeks sessions of halotherapy[12].

In three randomly selected cities in Southern Poland, Joana et al. conducted research on 303 individuals between the ages of 18 and 51. For the investigation, the author created a validated questionnaire. According to the data, 94% of respondents were aware of the existence of salt caves. 96 of the participants had participated in at least three salt cave sessions. While 39% of the men sought these sessions for both therapeutic and relaxation goals, 57% of the women did so for therapeutic reasons.

Men and women said that the sessions, which lasted 6 to 12 weeks, were effective in curing sinus, larynx, and throat issues. Only women stated that they attended these sessions due to depression. Individuals who tried this therapy reported feeling better overall after attending salt cave sessions. [13].

Ronen Bar and colleagues conducted extensive study on childhood asthma, a chronic inflammatory illness that necessitates continual anti-inflammatory medicine. Notably, mild asthmatic children aged 5 to 13 years are not currently receiving anti-inflammatory medication. To investigate potential remedies, the researchers randomly assigned patients to one of two groups: those who had access to a halogenerator in the salt room and those who did not. The intention was to examine the impact of salt room therapy on the asthma quality of life survey. There were 14 sessions in the therapy, which lasted for 7 weeks. In the trial, there were 26 patients in the control group who were placed in the salt room without a halogenerator, compared to 29 patients in the treatment group who were placed in the halogenerator-equipped salt room. According to the data, mild asthmatic children benefited more from salt rooms fitted with halogenerators. [14].

18 patients with bronchial asthma (12 with mild and 6 with intermediate disease) were examined by Gorbenko et al. before and after halotherapy for responsiveness of the airways. Halotherapy reduced bronchial hyperreactivity in 2/3 and 1/2 of the patients after 8 weeks, 12 sessions, consequently[15].

The effectiveness of salt chamber therapy as an additional therapy for reducing the need for inhaled steroid medication in asthma patients with bronchial hyperresponsiveness was examined by J. Hedman et al. The study comprised 32 asthma patients who responded to a histamine inhalation challenge with bronchial hyperresponsiveness. These patients were randomly assigned to one of two groups: 17 underwent active treatment, which included the use of a salt generator to introduce salt into the room, and 15 received a placebo treatment. Five times a week, a 40-minute salt chamber treatment was administered. The results showed that 15% of patients in the active treatment group had an increase in forced expiratory volume, while there was no meaningful change in the placebo group. As a result, adding salt chamber treatment effectively reduced bronchial hyperresponsiveness in asthma patients, allowing for a lower inhaled steroid dosage. [16].

Catalina et al. was studied effect of giving athletes to saline aerosols to improve their sports performances as well as breathing system. A sample of 12 middle-distance runners, aged between 14 and 16, were included in the study. During study, we would be detecting vital capacity (VC) and peak expiratory flow (PEF) of lungs. After salt therapy for 21 days, an analysis was conducted. The first day, saline aerosols were exposed to athletes for 20 minutes; the second day, for 25 minutes; and up to 60 minutes each day. Saline aerosol exposure for the previous 4 days was cut by 5 minutes, avoiding treatment interruption right away. After 21 sessions, each participant recovered, the individuals breathed regularly while relaxed, and an increase in respiratory volumes (VC, PEF) was seen in all of the

subjects. Additionally, improvements in respiratory mechanics and a rise in arterial blood oxygen saturation were noted[17].

Lazarescu et al. carried out a survey-based study in an underground salt mine to investigate the possibilities of speleotherapy or halotherapy. 18 people with bronchial asthma and chronic bronchitis (16 adults and 2 infants aged 6 to 13 years) participated in the study. 14 of these individuals had bronchial asthma as well as allergic rhinitis. A control group of four patients with bronchial asthma and chronic bronchitis were given in-home medication therapy but no speleotherapy or halotherapy. The findings demonstrated that bronchial asthma and chronic bronchitis patients who received halotherapy treatment experienced an anti-inflammatory response[18].

In a study of 124 individuals with various respiratory disorders, Abdrakhmanova et al. found that after spending an hour in a salt room each day for 15-20 days, the majority of participants' lung physiology significantly improved[19].

Similarly, in a different trial with 49 patients with chronic obstructive bronchitis, Valedeva et al. observed similar outcomes. Halotherapy has been shown to increase local immunity, free radical oxidation, and lung function.[20].

Richard Zagrobelny in 1966 examined infants at a hospital in Jerusalem. Doctors performed a series of tests on 38 infants between two and twelve months old. All are infected by various respiratory problems. The research showed that negative ions without any other treatment look to cure attacks of asthma and bronchitis more quickly than drugs. They also noticed that there were no adverse side effects found when treating such children with drugs[21].

Garavello et al. was studied halotherapy in children with asthma. He worked on asthma patients for 2 years involved 24 children in between five months to 18 years old. The process of halotherapy was performed in salt chambers where halogenerator generates aerosol salt particles. These salt particles were inhaled by children. The halotherapy indicated good results in patients with asthma and chronic obstructive bronchitis diseases and also displayed less use of drug prophylaxis. Consequently, children that had asthma and obstructive bronchitis 7 out of 24 restricted use of drug prophylaxis after halotherapy and no symptoms had appeared in later year. In 9 out of 24, halotherapy minimized use of drug prophylaxis and again there was no symptoms had appeared in later year. The best results observed on children were motivated and required more clinical studies so that to understand procedures that gives positive effects[22].

Oprita et al. studied 393 patients whose asthma or chronic obstructive lung disease was severe. 189 patients received standard care, including inhalatory corticosteroids, oxygen, and salt therapy for 204 more patients. After every 20 min oxygen saturation and respiratory rate of patients were evaluated. The level of respiratory gases was also observed after an hour. Generally, at start of salt therapy older age

patients were in severe conditions with more CO2 level and less O2 saturation. Additionally, after 1st hour all parameters were improved in patients treated with salt therapy. The oxygen saturation level were increased and partial pressure of carbon dioxide decreased[23].

Abdullaev et al. was studied efficiency of speleotherapy in children. He was used to stay 216 children with bronchial asthma in salt caves for 2 hours each day for 12 weeks. Evaluation of clinical and physiological parameters indicated that finest consequences were accomplished in patients of mild-to-moderate allergic asthma. The researchers suggested speleotherapy as an add on therapy to control childhood asthma[24].

Horowitz et al. was investigated 193 patients with chronic bronchitis. He noticed remarkable clinical results with dry salt therapy. As a result, over placebo 85% of mild and moderate cases, 75% in severe cases, and 97% in chronic bronchitis were recovered effectively with salt aerosols therapy after treatment session of 12-16weeks[25].

Beamon et al. was established three randomized clinical tests of speleotherapy in 126 children suffering from asthma. The two groups were showed best results with improved lung functions after speleotherapy but one group were required further improved methodological quality of speleotherapy. As a consequence, researchers assumed that more randomized tests were needed to get a reliable result whether this salt therapy is beneficial for treatment of chronic asthma in this population or not[26].

Simionka et al. was studied 55 patients with bronchial asthma showed a decrease number of T-lymphocytes and their IgA content. The cause of decrease in number of T- and B- lymphocytes were linked with presence of large number of cortisol-resistant lymphocytes. As a result, after 8–12 weeks of speleotherapy sessions, he saw a rise in T-lymphocyte numbers, control of B-lymphocyte numbers, an increase in immunoglobulin A levels, and a relative decrease in cortisol-resistant lymphocyte numbers[27].

Crisan et al. was conducted double-blind and randomized test for four months. He observed 128 patients with 76 asthma patients and 52 COPD stages II and III patients after first interview. We informed patients to utilize salt-inhaler 20min daily. We examined lung function test parameters (forced vital capacity, forced expiratory volume 1 and peak expiratory flow). Consequently, study showed an enhancement of all lung function test parameters after 1st month of treatment with salt therapy in contrast placebo. The final data indicated enhancement in Forced Vital Capacity by 4%, Forced Expiratory Volume1 by 14% and Peak Expiratory Flow parameter by 25%. All these parameters revealing remarkable improvement in condition of asthma patients. He concluded that salt aerosols looked to be more effective in contrast placebo when halotherapy utilized as an add on therapy with medication[28].

Chapter 3

3. Methods and Materials

I. Clinical Based Study

i. Survey Based Study of Human Specimens

3.1. Study Design

A cross sectional survey based and experimental study was performed. The survey-based study included 107 participants, of whom 8 were chosen for an experimental trial to determine the impact of salt therapy on asthmatics. A CBC (Complete Blood Count) was also conducted to determine the involvement of pulmonary immune cells in asthmatics for diagnostic purposes. **Area of Study**

The data of patients for survey was collected from Khewra Sanatorium from 2007 to 2020 after approval from PMDC (Pakistan Mineral Development Corporation) head office 13, H-9 Islamabad by General Manager (HR&EA) Mr. Muhammad Ilyas. The data of subjects were kept confidential and used only for study purposes. The experimental study on 8 subjects was conducted in Khewra salt mines sanatorium.

3.2. Participants Recruitment and Data Collection

Out of 653 patients, complete records of only 175 patients were available. 175 patients were contacted, and only 107 showed their willingness to be part of this study. The details of study and its importance was explained to each participant. The medical records of all participants who volunteered were reviewed. We used inclusion exclusion criteria for selection of participants[29]. We obtained consent from all participants and asked them to fill out a questionnaire containing socio demographic information (age, sex, reviews about salt therapy, recovery period). Data was analyzed in form of tables and graphs. More than half of the participants were males (67) and 40 were females. The incidence of asthma was 53.3% in children of age 2 months to 15 yrs. Table 1 shows demographic details of the participants.

Table 1. Demographic details of the participants

Parameters		Participants (N=107)
Gender	Male	67
	Female	40
Age	2mon-15yrs	57
	Age15-25	13
	Age26-35	17
	Age36-45	14
	Age46-55	6
Marital Status	Married	62
	Unmarried	45
Smoking Status	Smoker	4
	Non-Smoker	101
	Ex-Smoker	2

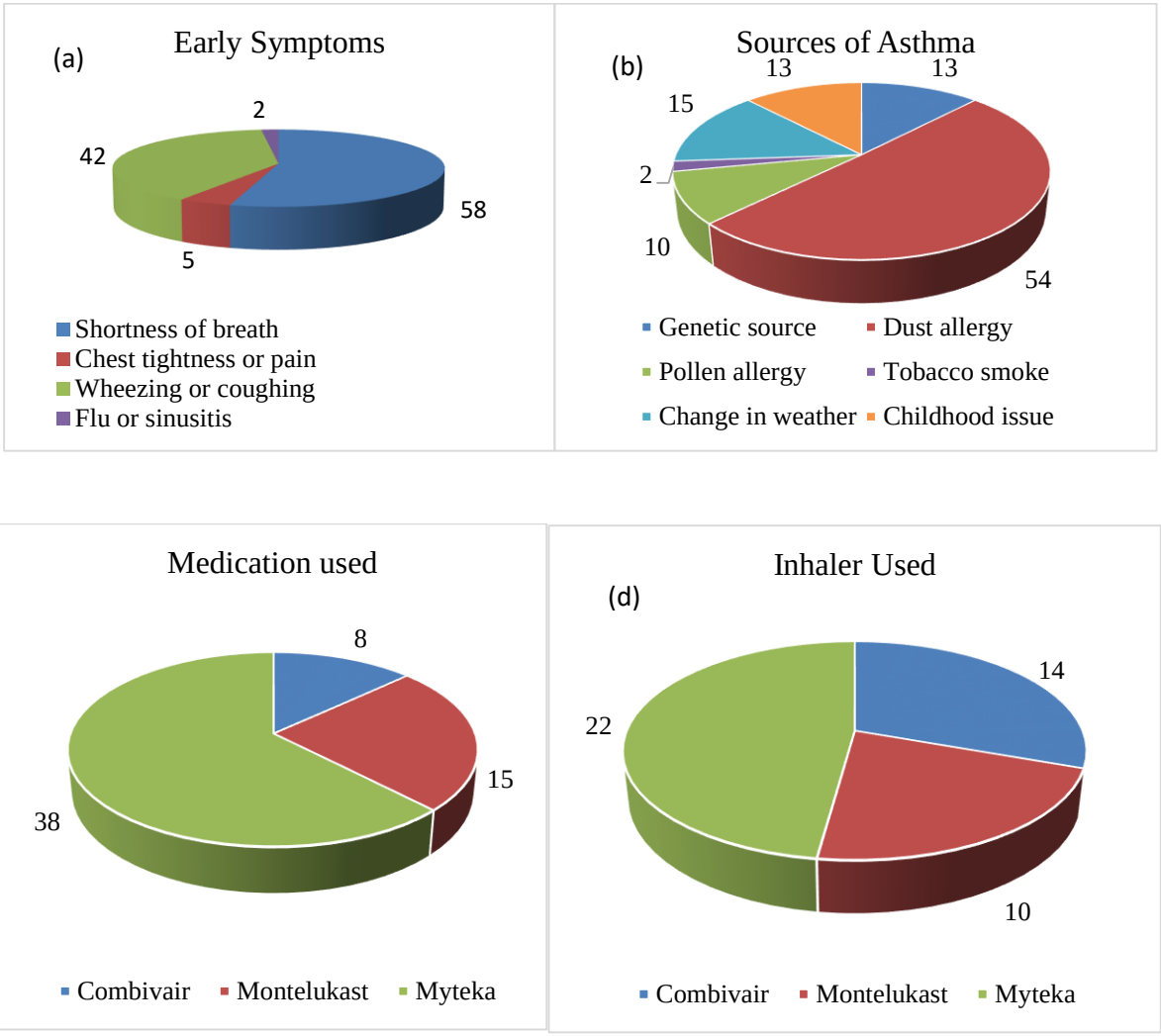


Figure 4. Data of participants in questionnaire-based study. (a) Shortness of breath was most common symptom in sample size of 107 asthma patients, (b) Dust allergy was the most frequent cause of asthma in

survey participants, (c) Majority of participants were used Medication, (d) Inhalers were also used by some participants in Survey based study.

ii. Experimental Based Study of Human Specimens

We randomly selected 8 participants out of 107, both male and female of all age groups to conduct an experiment on diagnostic test for asthma. CBC test was used to check role of pulmonary immune cells in asthmatics. Before experimentation, blood sample was collected, and CBC test was performed on all participants and their reports were obtained. Blood sample collection was done by a trained medical laboratory assistant. Participants were then asked to stay in Khewra salt mines sanatorium for 10 hours for 10 days. After 10 days, blood samples of all participants were collected again, and CBC was performed. Analysis was made based on immune cells count including platelets, eosinophil, monocytes, neutrophil and lymphocytes.

II. Vet Lab--Animal Based Study

3.3. Experimental Based Study of Wister Rats

3.3.1. Chemicals

In our study different chemicals were used for induction and treatment of asthma in Wister rats i.e., Alum (hydrated double sulphate salt of aluminum), Phosphate buffered saline (PBS) (a non-toxic solution), Ovalbumin (OVA), Saline solution, pink salt, Ethanol (de-contaminant), Chloroform and 10% neutral formaldehyde buffer (dissolved in PBS). Alum and Phosphate buffered saline (PBS) were purchased from Sigma-Aldrich USA. Ovalbumin was separated from egg white using standard protocol. Wister female rats were purchased from ASAB (Atta-ur-Rahman School of Applied Biosciences), National University of science & technology (NUST), Islamabad. Deionized water was used throughout study. Certeza NB 605 – Nebulizer (Nebulizer chamber capacity: 10ml max, Nebulization rate: 0.27 ml/min, Particle size: 3µm) was purchased from Iqbal & Son Enterprises.

3.3.2. Separation of Ovalbumin from Egg white

The egg yolk was separated from egg white by cracking egg and keeping yolk inside. The egg white was collected in a suitable container/beaker. About 100mL distilled or deionized water was added to egg white and stirred until a white precipitate formed. These were egg globulins. The mixture was centrifuged at 4000rpm for 6 minutes and poured off and supernatant (ovalbumin) was saved [30, 31].

3.3.3. Design an Animal Model for Asthma Study

3.3.3.1. Animals

35 Wistar female rats weighing 100-140g and aged 4-6 weeks were utilized in this study. The rats were maintained in separate cages with free access to a standard pellet diet and water ad libitum. They were

kept at a constant temperature of 22 ± 1 °C, a relative humidity of 55 ± 5%, and a 12/12h light/dark cycle. To perform serological study, rats were anaesthetized using chloroform. The regulation of good laboratory practice established by the US FDA (Food and Drug Administration) in 1978 concentrated on rat handling and care [32](Farooq & Fatima Rana, 2020).

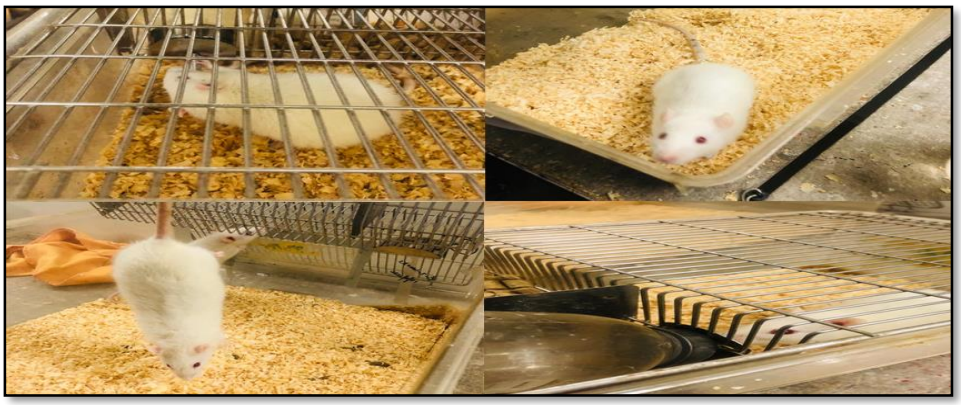


Figure 5. Female Wister Rats

3.4. Treatment Design

A. Positive Control/Disease Group

A set of five diseased rats were isolated and assigned tag as positive control (disease group). This group of rats was left untreated throughout the experiment, and survived rats were sacrificed at the end of experiment for immune cells analysis. Body and lung weight were noted.

B. Negative Control/Normal group

A set of five diseased rats were isolated and assigned the tag as negative/normal control. Throughout the entire procedure, this group of rats was not subjected to any adverse or allergic reactions and the survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted.

C. Saline solution Treatment Group of 0.5%

Five rats were placed in this group. Saline solution at the dose of 0.5% was inhaled by nebulization for the duration of 2 weeks. The survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted**.**

D. Saline solution Treatment Group of 1%

Five rats were placed in this group. Saline solution at the dose of 1% was inhaled by nebulization for the duration of 2 weeks. The survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted.

E. Saline solution Treatment Group of 3%

Five rats were placed in this group. Saline solution at the dose of 3% was inhaled by nebulization for the duration of 2 weeks. The survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted.

F. Saline solution Treatment Group of 5%

Five rats were placed in this group. Saline solution at the dose of 5% was inhaled by nebulization for the duration of 2 weeks. The survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted.

G. Saline solution Treatment Group of 6%

Five rats were placed in this group. Saline solution at the dose of 6% was inhaled by nebulization for the duration of 2 weeks. The survivors were sacrificed at the end of the experiment for immune cells testing. Weights for the body and lungs were noted.

3.5. Asthma Induction

The rats were initially released for two weeks (acclimatization phase). A total of 35 rats were split into two groups. 5 rats were used as a negative control, with no adverse reactions during entire experimentation, whereas 30 rats were subjected to allergic compounds by nebulization and then divided into one positive control group and five treatment groups, each with five rats. For consecutive 15 days, per rat dose inhaled was 0.0015g Alum, 0.01 µl Ovalbumin with 3ml of PBS. The purpose of ovalbumin exposure was to significantly damage lungs and to shorten the time needed to induce asthma. Ovalbumin (OVA) induces asthma with characteristic features such as airway inflammation, airway hyperresponsiveness (AHR) and airway remodeling (with increased mucus secretion of epithelial goblet cells)[33].

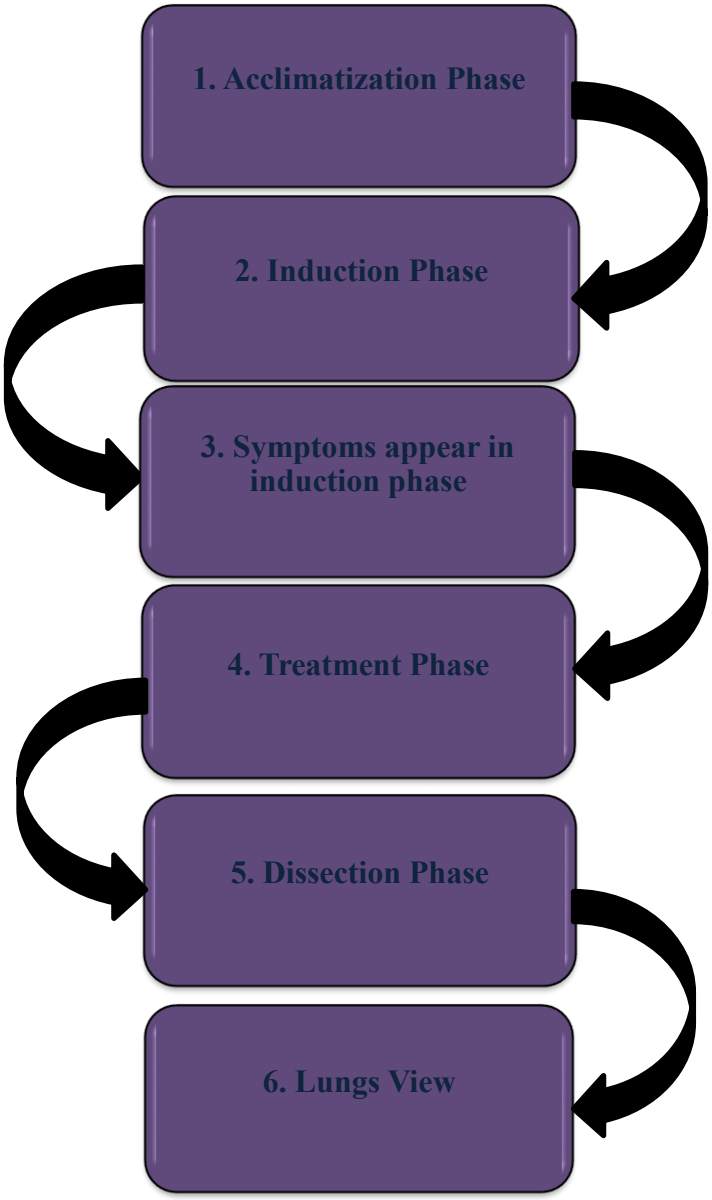


Figure 6. Diagrammatic and schematic presentation of research layout.

3.6. Physical Parameters of Rats

Different conditions like body weight, lung weight, nasal itching, sneezing and wheezing were observed.

3.7. CBC test for Immune Cells Analysis

Blood was extracted from the heart of Wistar rats for CBC (Complete Blood Count) test according to manufacturer's guidelines.

3.8. Histological Examination

After two weeks, diseased rats were sacrificed, the lung was harvested. The size, shape, texture and color of diseased lungs were obtained from lung tissue at the same time, fixed by 10% neutral-balanced formalin solution, 4um serial sections were obtained, followed by paraffin embedding. Subsequently, HE (Hematoxylin and Eosin) staining was carried out to observe the structural changes of lungs tissue which can be observed in histopathological slides[34].

Chapter 4

4. RESULTS

4.1. Statistical Analysis of Survey Based Study

Table 2 shows 45 participants experienced normal breathing after going through salt therapy, 26 participants had a relief from coughing whereas 20 of participants had no effect. 96 of survey participants only had one session of 10 days in a year. 66 of participants' condition improved after therapy. 18 showed a satisfactory response. 23 of the survey participants did not experience any improvement in their condition after therapy sessions. 42 of them had 70%-80% recovery rate and 23 participants had completely recovered after salt therapy.

Table 2. Percentage efficacy of participants for questionnaire-based study

Efficacy of salt therapy	Patients (N=107)
30-40%	27
50-60%	15
70-80%	42
100%	23

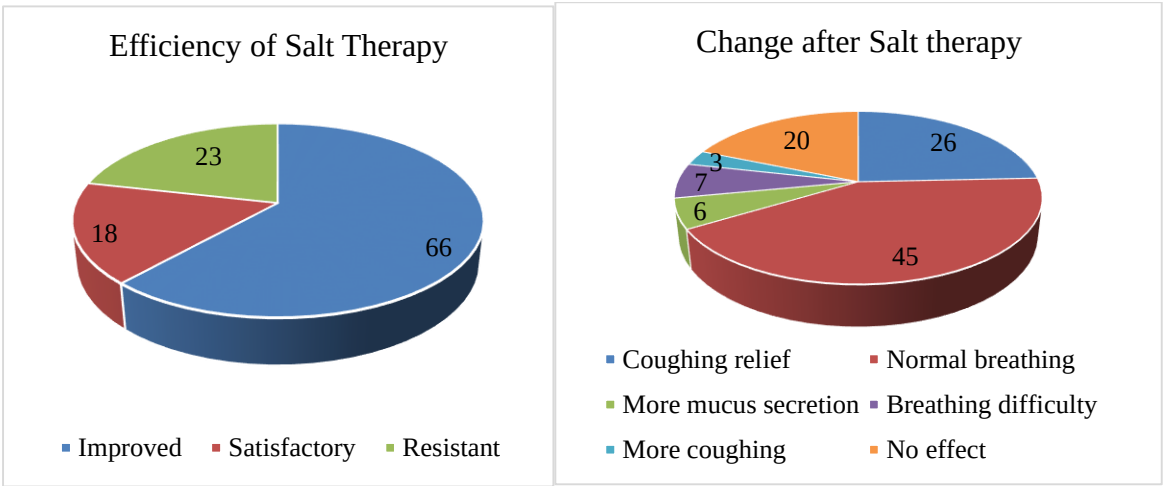


Figure 7. After effects of Salt therapy in participants of survey-based study

4.2. Experimental Based Human Specimens Data Analysis

The data of 8 participants who underwent salt therapy of 10 hours for 10 days were analyzed. The number of immune cells in blood of participants were checked before and after treatment (Table 3). It can be observed that after salt therapy count of immune cells decreases in all 8 patients. To check the significance of these results we applied paired sample t-test which is discussed in next section.

Table 3. Number of immune cells before and after salt treatment

No. of patients	Platelets		Eosinophil		Monocytes		Neutrophils		Lymphocytes	
	B	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**
AB01	350	303	7	4	12	6	78	61	48	31
AB02	184	154	9	4	13	6	57.1	53	42	37
AB03	250	170	12	4	18	5	72	55	53	36
AB04	300	259	7	6.5	15	3.4	82	69.6	55	42
AB05	256	127	8	3	17	6	88	56	56	30
AB06	250	134	8	2	13	4	83	70	50	24
AB07	635	377	12	2	18	2	92	68	54	35
AB08	450	350	10	1.6	13	6.9	78	68	53	32

B= Before A= After

4.2.1. Paired Samples Test

Based on our study design to check if salt therapy was effective in treating asthma patients or not, we formulated two hypotheses: i) Null Hypothesis: Salt therapy is not effective for asthma; there is no difference between means of immune cells count before and after salt therapy. ii) Alternative hypothesis: Salt therapy is effective for asthma, that is, there is a significant difference between means of immune cells number before and after salt therapy. The results show a significant difference in means of all immune cell counts before and after salt therapy treatment. P value of ≤ 0.05 is considered significant. As p value in case of all 5 immune cell numbers are less than 0.05, we accept our alternative hypothesis and state that salt therapy is an effective treatment for asthma patients (figure 8).

4.2.2. Graphical Representation of Individual Asthmatics

Figure 3 shows graphical representation of results of 8 patients described in Table 3.

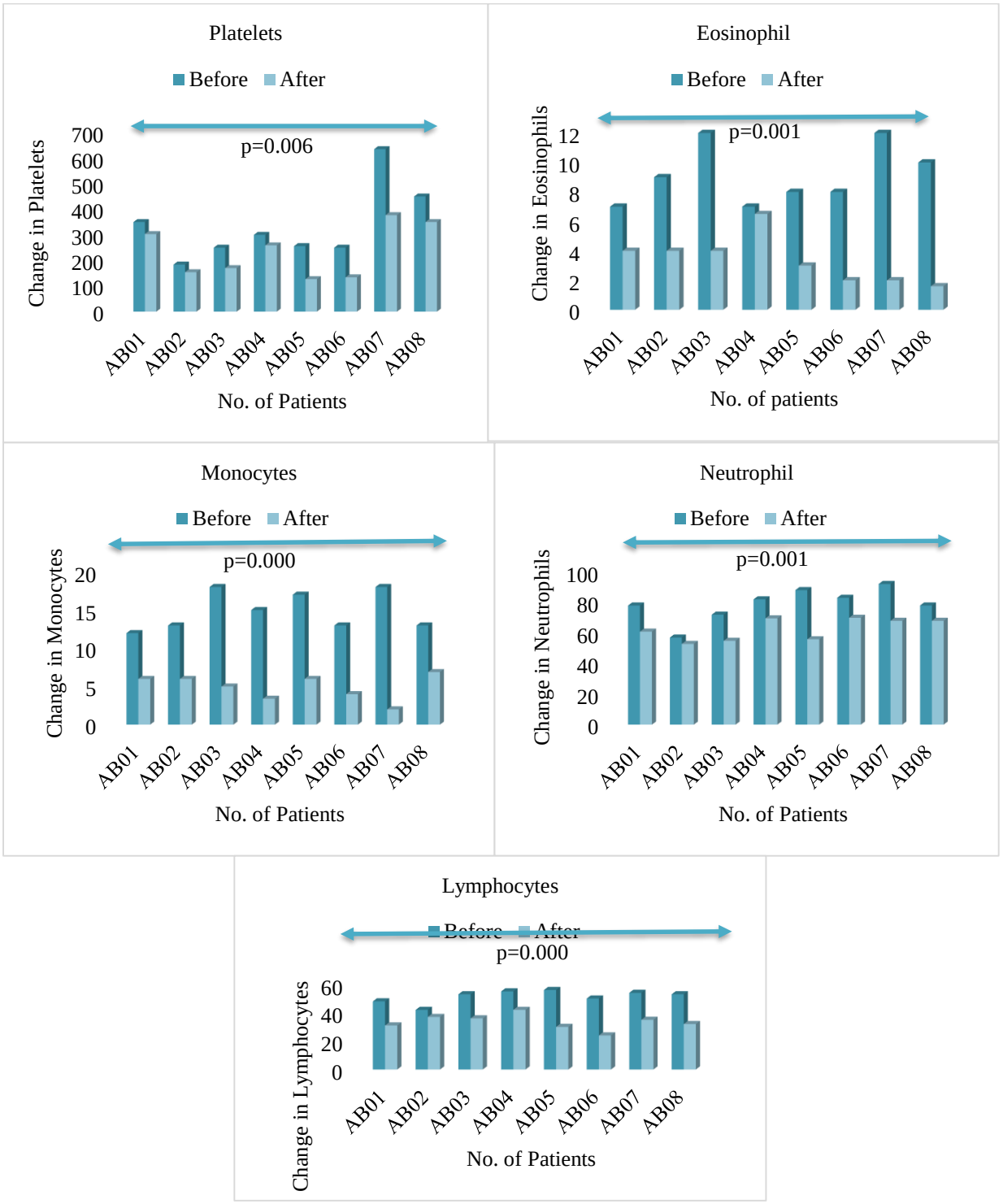


Figure 8. Asthmatic patient immune cells number before and after natural salt therapy treatment.

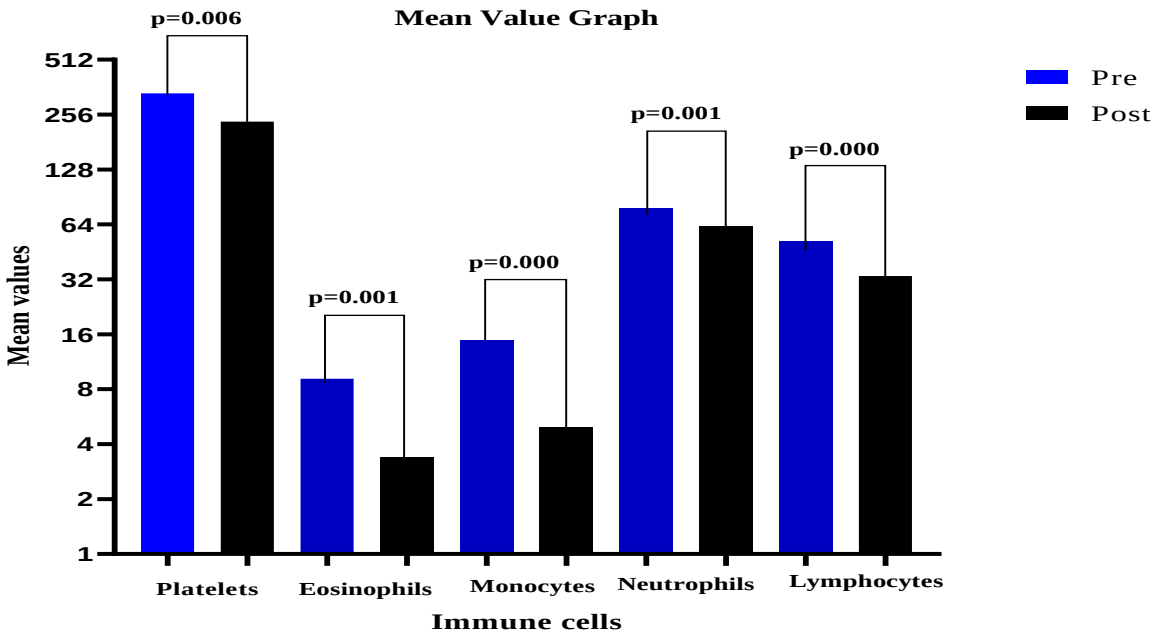


Figure 9. Mean value graph for each immune cell before and after salt therapy showed significant difference.

4.3. Mice Based Data Analysis

This section provides data analysis of experiment conducted on mice. Table 4 shows immune cells count of normal and diseased rats. It also shows the number of immune cells in treatment groups when exposed to various salt aerosols concentrations (0.5%, 1%, 3%, 5%, 6%). Figure 10 shows the graphical representation of comparison of immune cell count in diseased and treatment groups.

Table 4. Immune cells in control, diseased and different treatment groups in rats

Groups		Immune Cell Count				
		elets	trophils	phocytes	nocytes	nophils
Normal		50	65	40	5	3
Diseased		00	89	52	8	7
tment salt aerosols concentration (%)	0.5	12	70	44	6	4
	1	00	74	46	1	1
	3	50	84	46	4	2
	5	92	94	56	1	1
	6	50	81	59	3	1

4.3.1. Histopathology of Lungs

The histopathology results confirmed the induction of asthma after two weeks. The scoring was done to study different stages of asthma (Table 5). The histopathology results confirmed the induction of asthma after two weeks. The scoring was done to study different stages of asthma (Table 5). Inflammatory cells infiltrate in submucosal layer of bronchi and muscles of airway walls thickened is observed (a, b in Figure 12) including thickened bronchial walls, alveolar membrane slightly thick on routine microscopy, mild edema in some alveoli, and moderate to severe inflammation in lung tissue of rats (c, d, e, f in Figure 12) are observed. These changes varied from normal lung histology results, thus confirming successful induction of asthma after 15 days.

4.3.2. Immune Cells Analysis of Diseased Mice

The serological analysis was conducted to check the functioning of lung. It showed a significant difference in lung immune cells in CBC test reports of rats treated with ovalbumin, alum and PBS solution (figure 10).

Table 5. Weekly comparison of Asthma of normal versus OVA/alum treated rats

Weeks	**0**	**1**	**2**	**3**	**4**
	-	-	-	-	+
Asthma	-	-	-	+	+
	-	-	-	+	+
	No Asthma: - - -,	**Moderate Asthma: - + +,**	**Severe Asthma: + + +**		

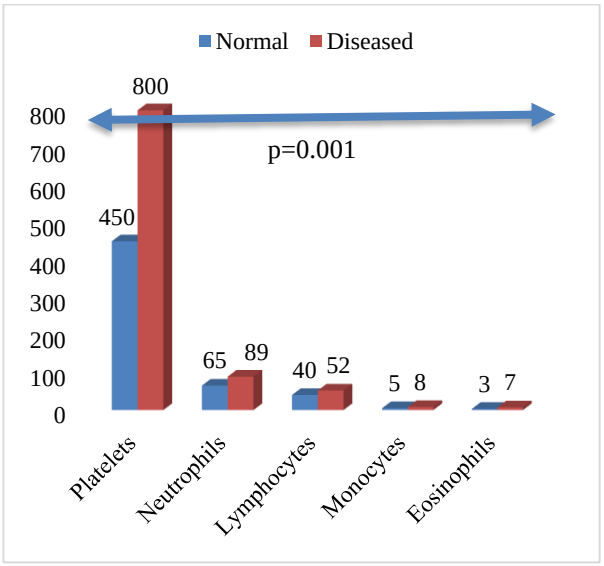


Figure 10. Comparison of blood count between control versus diseased asthmatic group of rats.

4.3.3. Body and Lung Weight

This study showed that change of body weight was significantly associated with asthma induction. During first two weeks of acclimatization, average weight of all groups was within range of 130-140gm. However, with the start of induction phase of asthma from week 3rd until the end of induction phase, a drastic change in body weight was observed. The body weight of rats treated with OVA, alum and PBS solution gradually decreased with time compared to normal rats. Moreover, lung weight significantly increased in 2nd week of asthma induction (figure 11). Weekly scoring level was checked according to scale organized for determining the stages of asthma (table 5). By end of first week of nebulization, various rats were observed scratching their bodies and nose. Bleeding from eyes of rats were also observed during disease induction whereas severe asthma was observed in 2nd week.

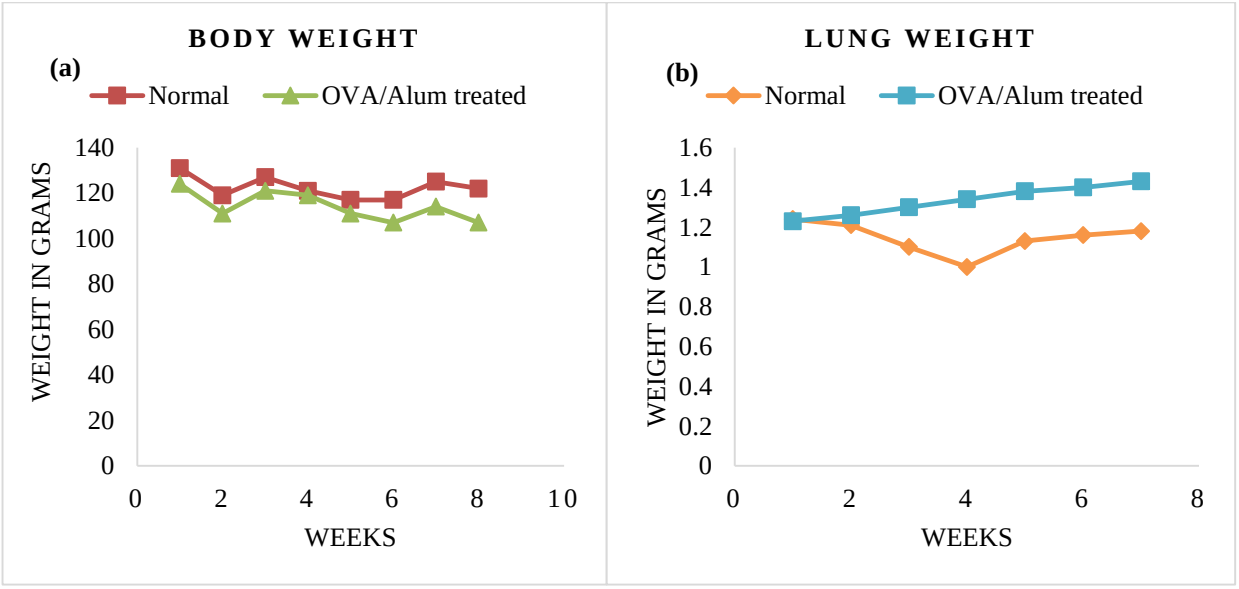


Figure 11. Weight analysis of normal versus OVA/alum treated rats from week one to week six. (a) Body (b) Lung.

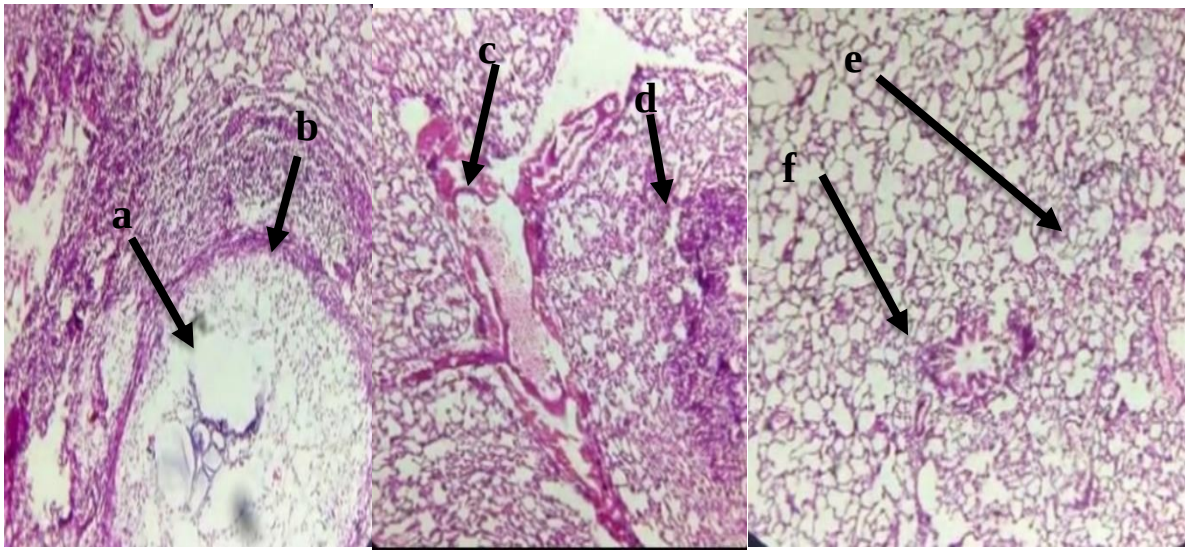


Figure 12. Histopathological analysis of lung during asthma induction. (a, b), indications of inflammatory infiltration and thickened muscles of airway walls (c, d), thickened lining of bronchi and alveolar membrane thickness (e, f), edema in some alveoli and severe inflammation in tissues of bronchi achieved in six weeks of asthma induction.

4.4. Treatment of Asthma induced Wistar Rats

The rats with successful induced asthma were subjected to treatment by intranasal inhalation of salt aerosols as 0.5%, 1%, 3%, 5% and 6% of pink salt solution for two weeks. The histopathological analysis, serological analysis, body and lung weight showed the effectiveness of salt aerosols in treating asthma induced rat model by comparing these results with that of normal rats.

4.4.1. Histopathological Analysis of Lung

The lung histopathological analysis is provided in figure 16. In lung tissue histology, the different treatment groups treated with intranasal salt aerosols concentration showed different results. The rats treated with 0.5%, 1% and 3% pink salt aerosols showed no inflammation in lung tissues, normal inflammatory cells in submucosal layer of bronchi, thin muscles of airway walls, normal alveolar membrane on routine microscopy, no edema in alveoli and normal intact bronchioles (a, b, c in figure 16. A, B, C) were observed. But the rats treated with 5% and 6% pink salt aerosols concentration showed abnormal changes in lung histology. Severe inflammation in walls of bronchi, the alveolar membrane and alveoli became disrupted and thickened bronchial walls were observed (d, e, f in figure 16. D, E).

4.4.2. Body and Lung Weight

This study showed that with the start of treatment phase from week 5th to the end of experiment the weight of body significantly increased in treatment groups but lung weight analysis showed significant decrease from that of diseased rats (Figure 13).

4.4.3. Serological Analysis of Treated Rats

The serological indices noticed after performing CBC test showed a significance difference between diseased and different treatment groups of rats. (Figure 14).

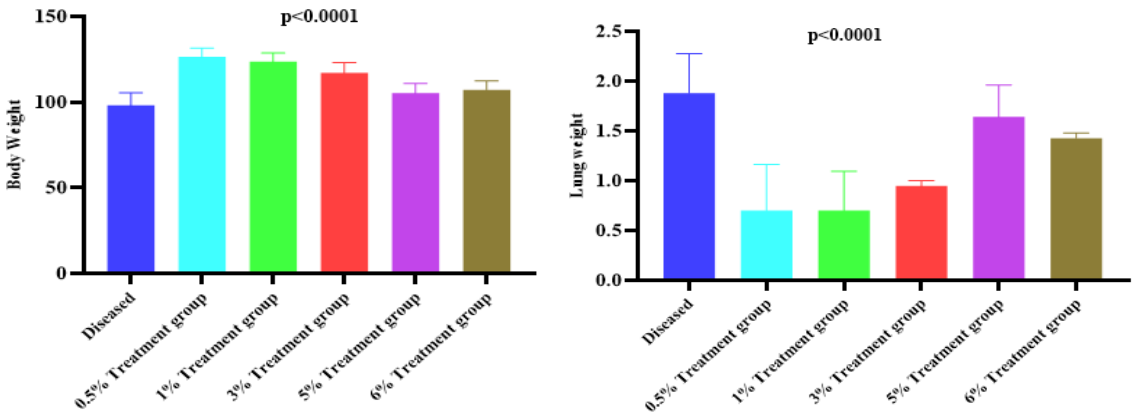
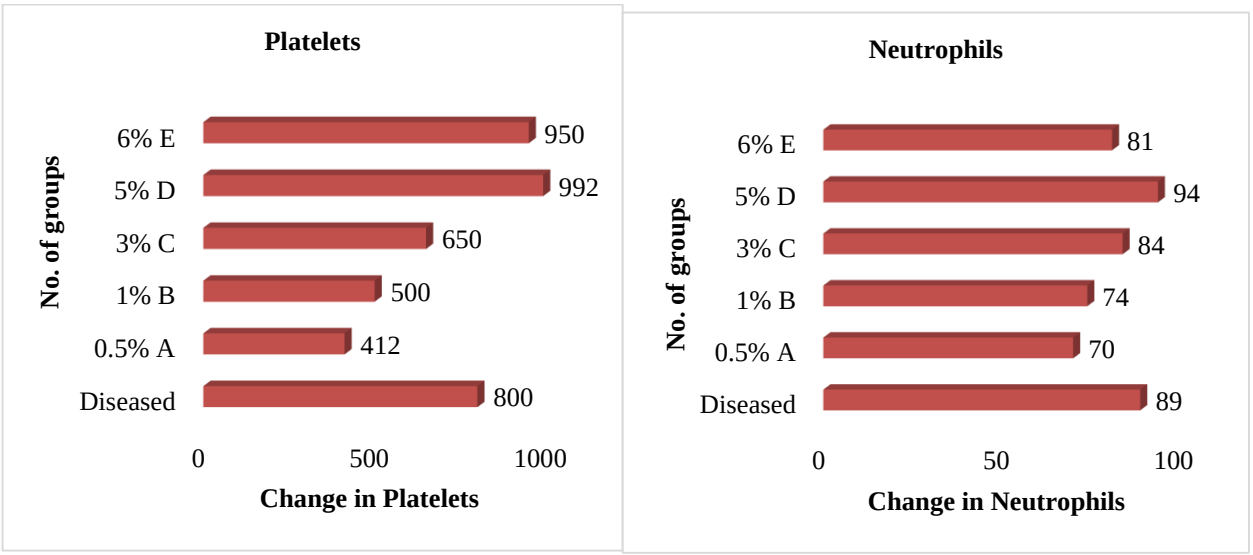


Figure 13. Body and lung weight of different treatment groups of percentage salt dose concentration of rats with diseased rats.



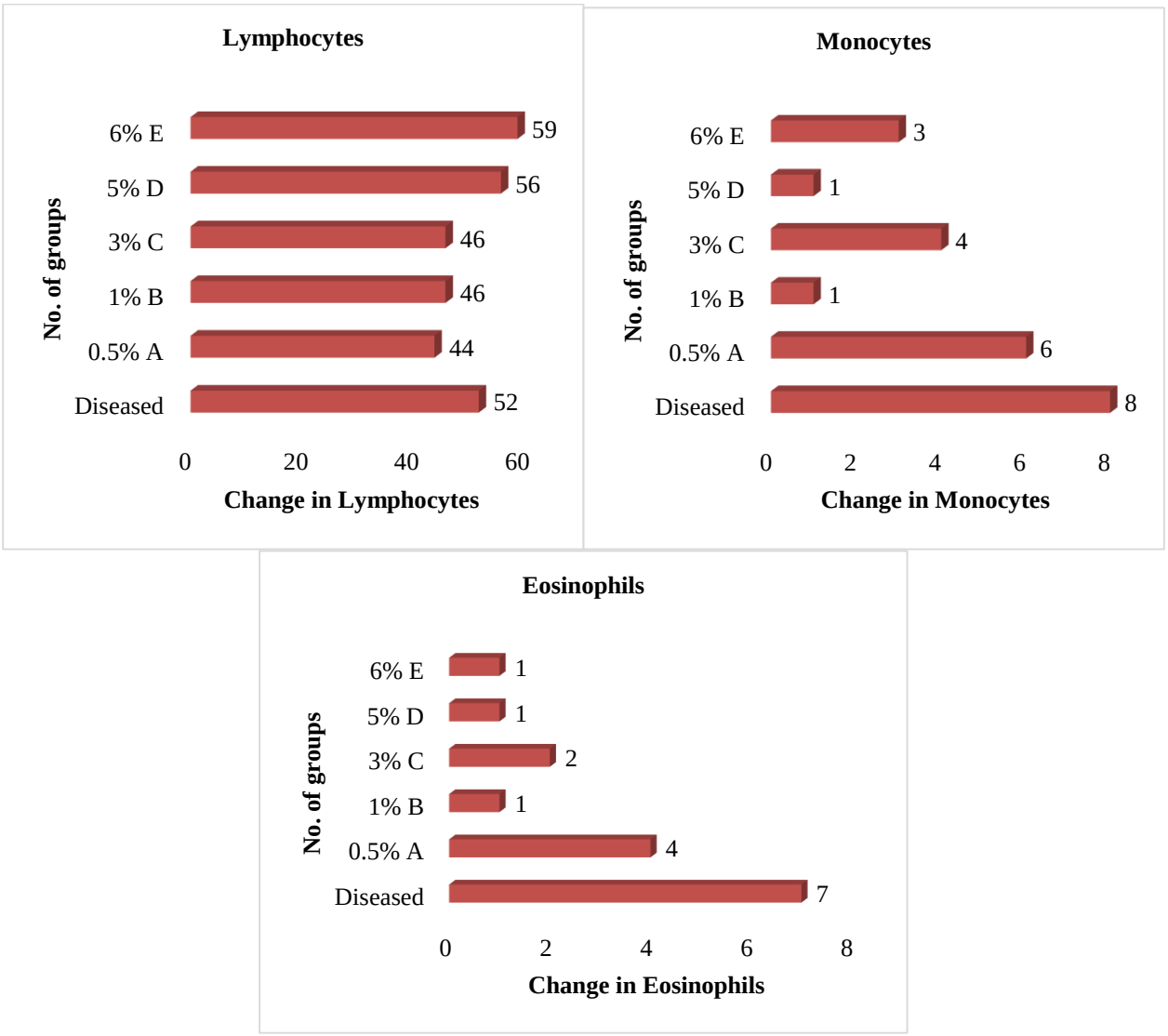


Figure 14. Immune Cells Analysis by using ovalbumin/Alum solution treated rats versus different dosage concentration of Salt aerosols treatment groups.

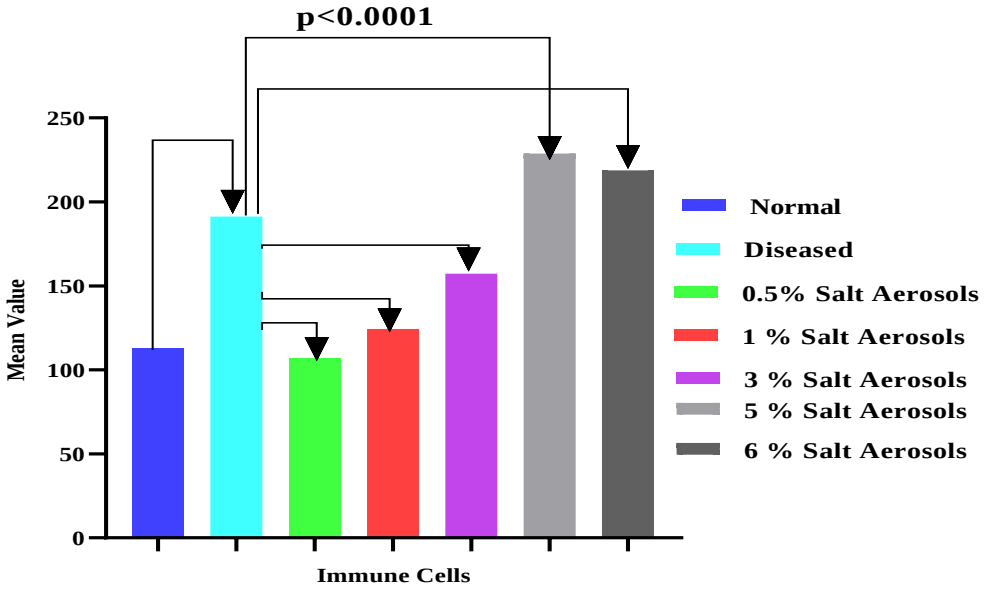


Figure 15. Mean value graph for immune cells analysis. Comparison of Normal/ Diseased; Diseased /0.5% salt aerosols treatment group; Diseased/1% salt aerosols treatment group; Diseased/3% salt aerosols treatment group; Diseased/5% salt aerosols treatment group; Diseased/6% salt aerosols treatment group. (Significant at <0.005)

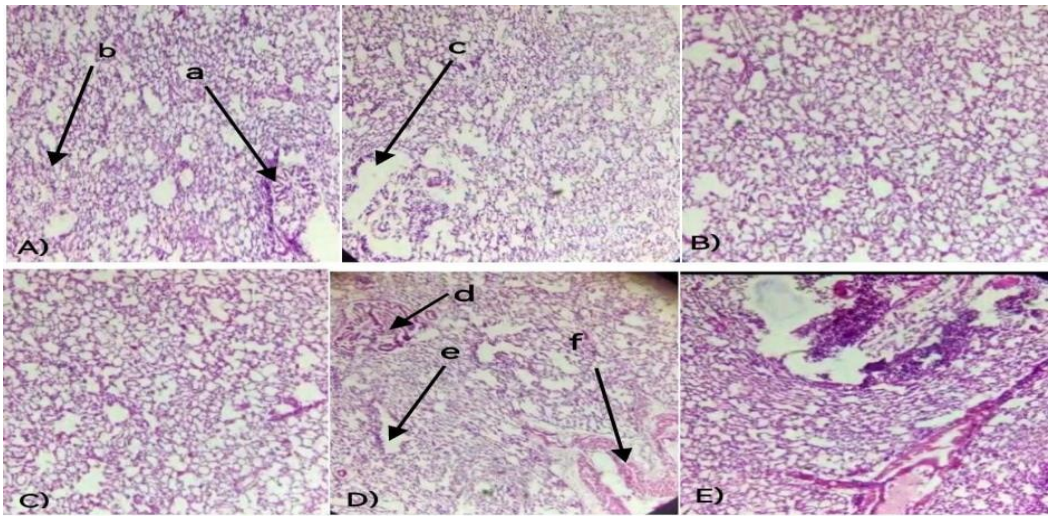


Figure 16. After treatment with intranasal pink salt aerosols. (A) 0.5% salt aerosols treated group, (B) 1% salt aerosols treated group, (C) 3% salt aerosols treated group, (D) 5% salt aerosols treated group, (E) 6% salt aerosols treated group.

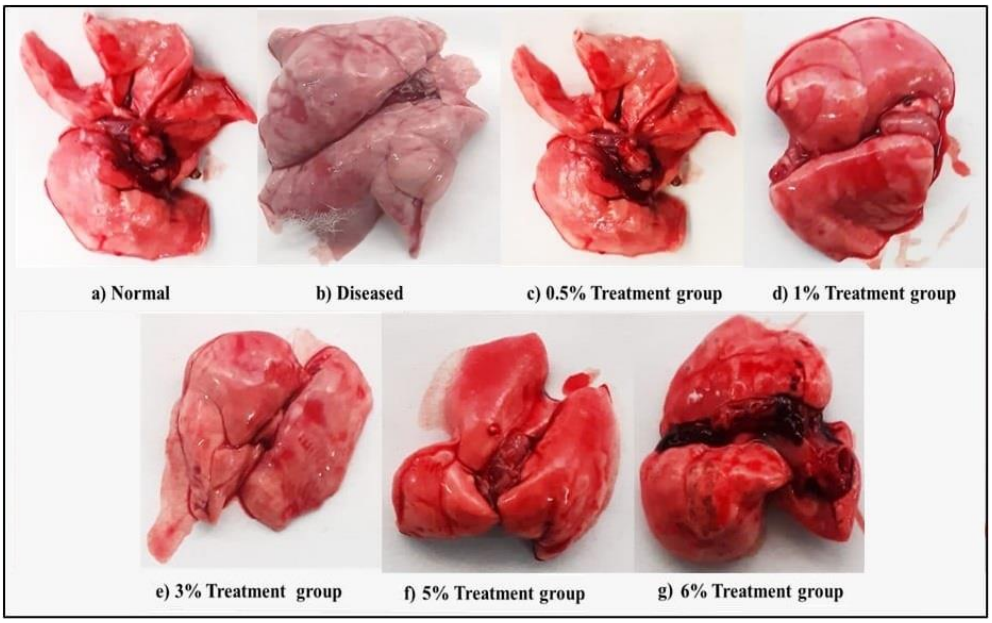


Figure 17. Lungs photographs after dissection

Chapter 5

5. DISCUSSION

Airway obstruction is the main feature of asthma, which is produced by a reduction in the airway's diameter. The decreasing airways diameter is resulted by chronic inflammation of the airway wall, and activation of immune cells such as eosinophil, neutrophils, lymphocytes, and monocytes. A complex relation between these immune cells and with neighboring cells such as platelets results to the development of asthma such as BHR. Moreover, in severe forms of asthma, airway obstruction does not always normalize by medication or inhaler. In such patients, addition of life long mucus plugs in smaller airways can be fixed airway obstruction or other mechanisms, including airway remodeling[28]. As specialist have identified that severe and persistent asthma occurs from heterogeneous conditions. These findings have helped to the development and testing of new classes of asthma treatments that focus on specific immune pathways. Developing therapies now allow specialists to give personalized medication to patients with severe asthma[29]. Recently, medications used for asthma control reduces airway inflammation and assist to lessen asthma symptoms; among these, inhaled corticosteroids (ICS) are the mainly used in the treatment of asthma, whereas quick-relief (reliever) or rescue medicines immediately ease symptoms that may originate acutely. It includes short-acting beta-agonists (SABAs) rapidly decrease airway bronchoconstriction (resulting relaxation of airway smooth muscles)[30]. But these medications have some side effects such as fungal infection, sore throat, decreased bone density in adults and cataracts. Other side effects included muscle weakness, high blood pressure, high blood sugar, aggression, depression and sleep disturbance[31]. In another clinical study, rats with mild to severe respiratory disorders results sniffling, dyspnea, ruffled hair coat and weight lose but lung weight increases due to thickness of airway walls and more mucus in airways. Major changes observed in rats affected with respiratory disorders are characterized by change in immune system function as neutrophils, plasma cells and lymphocytes results chronic bronchitis[32]. Therefore, scientists are introduced drug free treatment method for asthma patients with no side effects. This therapeutic method is called speleotherapy or dry salt therapy. The therapeutic effects of dry salt therapy are beneficial for health mostly for respiratory disorders and also cheap and very useful therapeutic method[33]. Previous research papers related to human and animal study also shows that salt therapy is an effective treatment for respiratory disorders with a specific focus on asthma but animal study on salt therapy is sufficient. Eighteen rats with wheezy or congested or for respiratory distress placed in a cage and treated with nebulizer of 2ml saline solution for 15mints each day. Saline with nebulizer thin the mucus secretion so that rats expel it easily and this saline solution also help to moisturize the mucus membrane to avoid irritation[34]. Therefore, we can say that naturally created salt environment is valuable for therapeutic

purposes. Chervinskaya et al. (1995) conducted a study in salt room on brochial asthma patients. They concluded that salt particles are effective for deep penetration in bronchi and improve the function of lungs[18].

After successful induction of asthma, the allergic effect of OVA/Alum with PBS solution were analyzed to validate the model. The histopathological, serological, body and lung weight results showed the significant changes from mild to severe asthma over the period of 2 weeks. Results were indicators of successful asthma model development by the end of second week.

Following treatment of diseased rats, the serological, histological, body and lung weight results showed significant improvement in groups treated with salt aerosols. Apart from that, treated groups that were treated with percentage concentration of 0.5%, 1%, 3% showed better results up to some extent. Therefore, for asthma disease, the use of low salt aerosols concentration is better choice, contrary to more salt aerosols concentration. In addition, to the route of administration for salt aerosols, the intranasal route has showed better results, although further research is required to study the effects of oral administration as well. As such, salt aerosols are found to be effective in treatment of asthma disease. Moreover, salt therapy has emerged as an advance treatment method for asthma. It reduces frequency of asthma attack and minimizes the use of medication. However, impact of salt therapy is not still researched sufficiently, which indeed demands resumption of research. Although our study gave positive and significant results. It seems that asthmatics could use salt therapy as a complementary treatment along with medication[35].

Asthma is explained as a hypersensitivity reaction in which immune system activates inflammatory reaction which harms airways in a response to protect the body from an allergen. It is a chronic condition, there are various methods used to improve symptoms and quality of life. This includes corticosteroids tablets or inhalers[27]. However, potent anti-allergic treatment is still awaited but as a natural remedy, salt therapy can be used for asthmatics. The therapeutic effects of dry salt therapy are beneficial for health mostly for respiratory disorders[28]. Because Himalayan pink salt has more mineral content that are functionally very useful for human body. Natural Himalayan salt mine environment is used to make patients inhale negative ions for treatment of many respiratory and pulmonary diseases like asthma[29]. The results of survey showed a positive response from patients receiving salt therapy. 42.07% had a normal breathing only after 1st week of therapy. 61.68% participants said that their condition improved after therapy. After salt therapy, most of the patients were completely recovered. Some patients continued to take medication after salt therapy but for short period of time. Many patients decreased the use of inhaler after the therapy. In case of experimental study on 8 patients, when paired t-test was applied on means of immune cell count before and after the salt therapy, the results showed a significant difference in table 3. We had used immune cells count parameter for asthma study because

immune system plays key role in triggering asthma symptoms. As a results, we can say that naturally created environment is valuable for therapeutic purposes and also regulating the immune system. But not enough previous research based on immune system was available for asthma study. Previous research shows that salt therapy is an effective treatment for asthma. Simionka et al. (1998) was studied 55 patients with bronchial asthma showed an increase number of T-lymphocytes and their IgA content. The cause of increase in number of T- and B- lymphocytes were linked with presence of large number of cortisol-resistant lymphocytes. Consequently, after speleotherapy session he observed an decrease in number of T-lymphocytes, regulation of the amount of B-lymphocytes[30]. Scientists at the University of Manchester has also explained salt has potential to regulate healthy inflammatory processes. In an animal study they investigated effects of salt therapy to decrease swelling[31]. Chervinskaya et al. (1995) conducted a study in salt room on brochial asthma patients. They concluded that salt particles are effective for deep penetration in bronchi and improve the function of lungs[24]. Chernenkov et al. (1997) results also concluded that salt therapy is an outstanding add on therapy for COPD patients with improved volume flow rate of lungs[32]. Lazarescu et al. (2014) also conducted survey-based study on asthma patients. They concluded that frequency of asthma attack was reduced in patients who received salt therapy sessions on a regular basis[33]. 303 locals (18–51 years old) from three randomly selected cities in southern Poland were studied by Joana et al. (2014) utilizing a validated author's questionnaire. It was discovered that the diseases evaluated to be treated by sessions (6–12 weeks) included issues with the throat, larynx, or sinuses in both women and men. Generally, those who was used this therapy felt better after sessions in salt caves[34]. Horowitz et al. (2010) was investigated 193 patients with chronic bronchitis. He noticed remarkable clinical results with dry salt therapy after treatment session[35]. In addition, more remarkable results are estimated in children and adults than in older people. Because children and adults have greater recovery rate and strong immune system than older people[36]. Salt therapy has emerged as an advance treatment method for asthma patients. It reduces frequency of asthma attack and minimizes the use of medication. However, impact of salt therapy is not still researched sufficiently, which indeed demands resumption of research. Although our study gave positive and significant results. It seems that asthma patients could use salt therapy as a complementary treatment along with medication[37].

Chapter 6

6. CONCLUSION

This study's objective was to evaluate the effectiveness of salt therapy as an asthma treatment. We conducted a survey based cross sectional study along with an experimental part to check the effect of salt therapy in asthma. The experimental study on human subjects gave significant results in immune cell (neutrophils, monocytes, lymphocytes, eosinophils and platelets) count of asthma patients. In conclusion, our study showed that salt therapy can be used as a treatment for asthma. For future studies, a large sample size and longer duration of therapy is recommended to avoid errors and get more accurate results. We further plan to perform the study in a more controlled environment keeping the impact of humidity and temperature under consideration and developing a standard protocol. Having the attention from the medical community, the need for more convenient access became a necessity, in order to conduct specific studies and do more specific research. Moreover, we also conducted experimental based study on mice models that gave significant results in immune cell (neutrophils, monocytes, lymphocytes, eosinophils and platelets) count of asthma induced rats. In conclusion, our study showed that salt therapy can be used as a treatment for asthma. For future studies, a large sample size and longer duration of therapy is recommended to avoid errors and get more accurate results. We further plan to perform the study in a more controlled environment keeping the impact of humidity and temperature under consideration and developing a standard protocol.

Chapter 7

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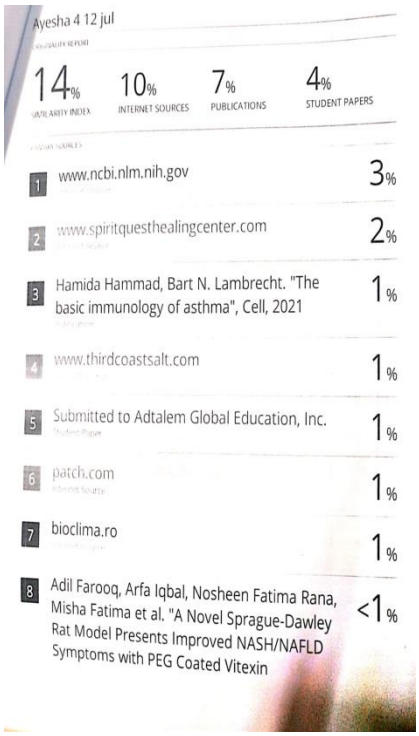

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