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# ASSESSING ASTHMATIC OUTCOMES BY STANDARD DRUGS USING STANDARD PROCEDURES

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## INTRODUCTION

**Definition:** Asthma is defined as a syndrome in which there is a recurrent reversible obstruction of the air ways in response to stimuli which are not themselves naxious .

**KEY POINTS:**

*Asthma is the major non-communicable disease (NCD).

*Asthma affected an estimated 262 million people in 2019 and caused 455000 death.

*Most asthma related deaths occur in low and lower middle income countries, where under diagnosis and under treatment is a challenge.

*WHO is committed to improving the diagnosis and treatment and monitoring of asthma to reduce the global burden of NCDs and make progress towards universal health coverage.

*Inflammation and narrowing of the small airways in the lung cause asthma symptoms, which can be any combination of cough, wheeze, shortness of Breath and chest tightness.

*Asthma is a long term condition affecting children and adults.[2]

**EPIDEMIOLOGY:**

●The 2003 Canadian Community health survey found that 8.4% of the Canadian population ≥ 12 years of age had been diagnosed with asthma, with the prevalence being highest among teens (>12%) [3]. Between 1998 and 2001, close to 80,000 Canadians were admitted to hospitals for asthma, and hospitalization rates were highest among young children and seniors. A population-based cohort study conducted in Ontario found that age and sex-

standardised asthma prevalence increased from 8.5% in 1996 to 13.3% in 2005, a relative increase of 55%[4].There are 2 major categories of asthma.

1.Allergic asthma.

2.Non – Allergic asthma.

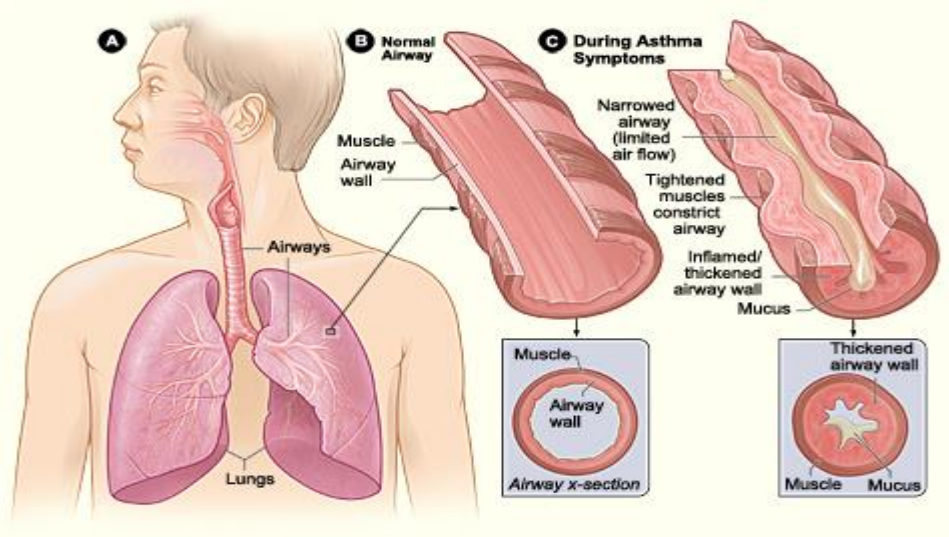



**Fig 1: Bronchial asthma pharmacology.**

## HISTORY OF ASTHMA:

Asthma was recognized in ancient Egypt and was treated by drinking an incense mixture known as kyphi.[5] It was officially named as a specific respiratory problem by Hippocrates circa 450BC, with the Greek word for painting forming the basis of our modern name. In 200BC it was believed to be at least partly related to the emotions, In the 12th century the Jewish physician- philosopher Maimonides wrote a treatise on asthma in Arabic, based partly on Arabic sources in which he discussed the symptoms, proposed various dietary and. Other names of treatment, and emphasized the importance of climate and clean area.

In January 2021, an appeal court in France overturned a deportation order against a 40- year-old Bangladeshi man who was a patient of asthma. His lawyers had argued that the dangerous levels of pollution in Bangladesh could possibly lead to worsening of his health condition, (or) even premature death.[6]

**Fig 2: History of asthma.**

## Symptoms:

1.Shortness of breath.

2.Chest tightness (or) pain.

3. Wheezing when exhaling, which is a common sign of asthma in children.

4. Trouble sleeping caused by shortness breathe, coughing (or) wheezing.

5. Frequent infection.

6. Anxiousness (or) panic.

7. Coughing especially at night, when laughing, (or) during exercise.

- Certain symptoms which include worsening of asthma condition are:

1.Frequent asthma attacks.

2.Worsening breathing difficulty with attacks.

3.Frequent use of inhalers.[7]

## CAUSES:

1.**Allergies:** Having allergies can raise your risk of developing asthma.

2.**Environmental factors:** People can develop asthma after exposed to things that irritate the airways. These substances include allergens, toxins, fumes and second or third-hand smoke. These can be especially harmful to infants and young children whose immune system haven't finished developing.

3.**Genetics:** If your family has a history of asthma or allergic diseases you have a higher risk of developing the disease.

4.**Respiratory infections:** Certain respiratory infections such as syncytial virus (RSP) can damage young children developing lungs.

5.Illness.

6.Overload physical movements.

7.Emotions including stress or laughter.

8.Non-Steroidal Anti-inflammatory drugs.

9.Due to some food and beverages.

10.Molds and cockroaches.[8]

## DIAGNOSIS OF ASTHMA:

The main tests used to help diagnose asthma are:

**1. Pulmonary function Test:** This test is done to find out how much can the lungs hold and how much air is taken in and given out by the lungs.

**2. Feno Test:** You breath into a machine that measures the level of nitric oxide in your breathe, which is sign of inflammation in your lungs.

**3. Spirometry:** You blow into a machine that measures how fast you can breathe out and how much air you can hold in your lungs.

**4. Peak flow Test:** You blow into a handheld device that measures how fast you can breathe out, and this may be done several times over a few weeks to see if it changes over times.

**5. Chest X-ray:** Chest x-ray is not needed to diagnose asthma. It is only needed only when the diagnosis is not clear or any complications are suspected.

**6. Physical examination:** Dyspnea, expiratory wheeze, accessory muscle movement, difficulty in feeding, talking, getting to sleep.

**7. Imaging Tests:** A chest x-ray can help identify any structural abnormalities or diseases that can cause or aggravate breathing problems.

**8. Allergy Testing:** Allergy test can be performed by a skin test or blood test. They tell you if you're allergic to pests, dust, or pollen. If allergy triggers are identified, your doctor may recommend allergy shots.[9]

## TYPES OF ASTHMA:

Asthma is broken down into two types based on the causes and the severity of symptoms. Health care providers identify asthma as:

- **Intermittent:** This type of asthma comes and goes so you can feel normal in between asthma flares.

*Intermittent asthma is a type of asthma where in a person has symptoms on no more than 2 days per week and nightly flares on no more than 2 nights per month.

* Intermittent asthma can range in severity and is usually treatable with medications.

* A person with intermittent asthma does not experience regular breathing difficulties, but may only have flare-up symptoms once every few months.

● **Allergic Asthma:** Allergic asthma is the most common type, affecting around 60% of people with asthma in the United States.

*Around 8 in 10 people with allergic asthma will also have another allergic condition, such as eczema, allergic rhinitis, food allergy.[10]

*Certain allergens in the environment can trigger allergic asthma.

**●Non-allergic Asthma:**

*It is also called as intrinsic asthma.

*This type of allergens does not require an allergen to trigger an attack.

*It is less common than allergic asthma, accounting for 10-30% of all asthma cases. It is more likely to appear in adulthood and affects more females than males.

*Experts believe that nonallergic asthma develops due to genetic and environmental factors.

**●Severe Asthma**:

* Severe asthma (OR) brittle asthma, effects around 4% of all adults with asthma.

*Experts consider to be severe when symptoms do not improve with standard medications. People with severe asthma may be likely to

→Have more asthma attacks than people with mild to moderate asthma.

→Spend more time in the hospital as a result of their asthma.

→Take steroid tablets long term.

**●Asthma in pregnancy:**

*Asthma is one of the most common diseases among pregnant women. Some experiences an improvement (OR) worsening of asthma symptoms during pregnancy, while others develop the symptom for the first time.[11]

*During pregnancy, it is important to work with the doctor to recognize, manage and treat asthma symptoms.

* Mucosa and laryngeal oedema may be mediated by the oestrogen hormone leading to rhinosinusitis in about 20% of pregnant women.[11]

*Without treatment, asthma may increase the risk of the following issues:

1.Pre-eclampsia, a serious medical indication.

2.Low birth weight.

3.Poor foetal growth.

## PATHOPHYSIOLOGY OF ASTHMA:

Pathophysiology of asthma involves the bronchial circulation my also play an important role in regulating airway caliber, since an increase in the vascular volume may contribute to airway narrowing. [12] Vascular balancing of the skin is used to measure the potency of topical steroids and a similar mechanism may be involved with the effect of inhaled steroids in asthma. Increased airway blood flow may be important in removing inflammatory mediators from the airway, and may play a role in the development of exercise-induced asthma.

Increased shear stress due to high expiratory pressure may lead to gene transduction and enhanced production of nitric oxide by type III (endothelial) NO synthase. Microvascular leakage is an essential component of the inflammatory response and many of the inflammatory mediators implicated in asthma produce this leakage. There is a Good evidence for microvascular leakage in asthma, and it may have several consequences on airway function, including increased airway secretions, impaired muco ciliary clearance formation of new mediators from plasma precursor (such as kinins) and mucosal oedema which may contribute to airway narrowing and increased airway hyper responsiveness.[13,14]

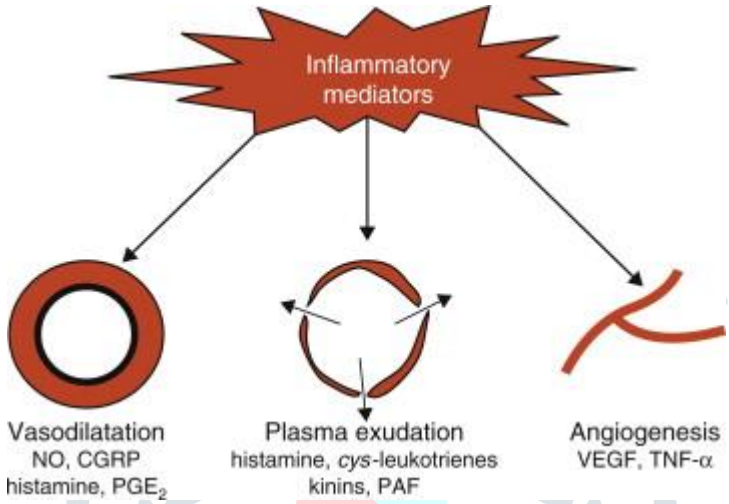



**Fig 3: Pathophysiology of asthma.**

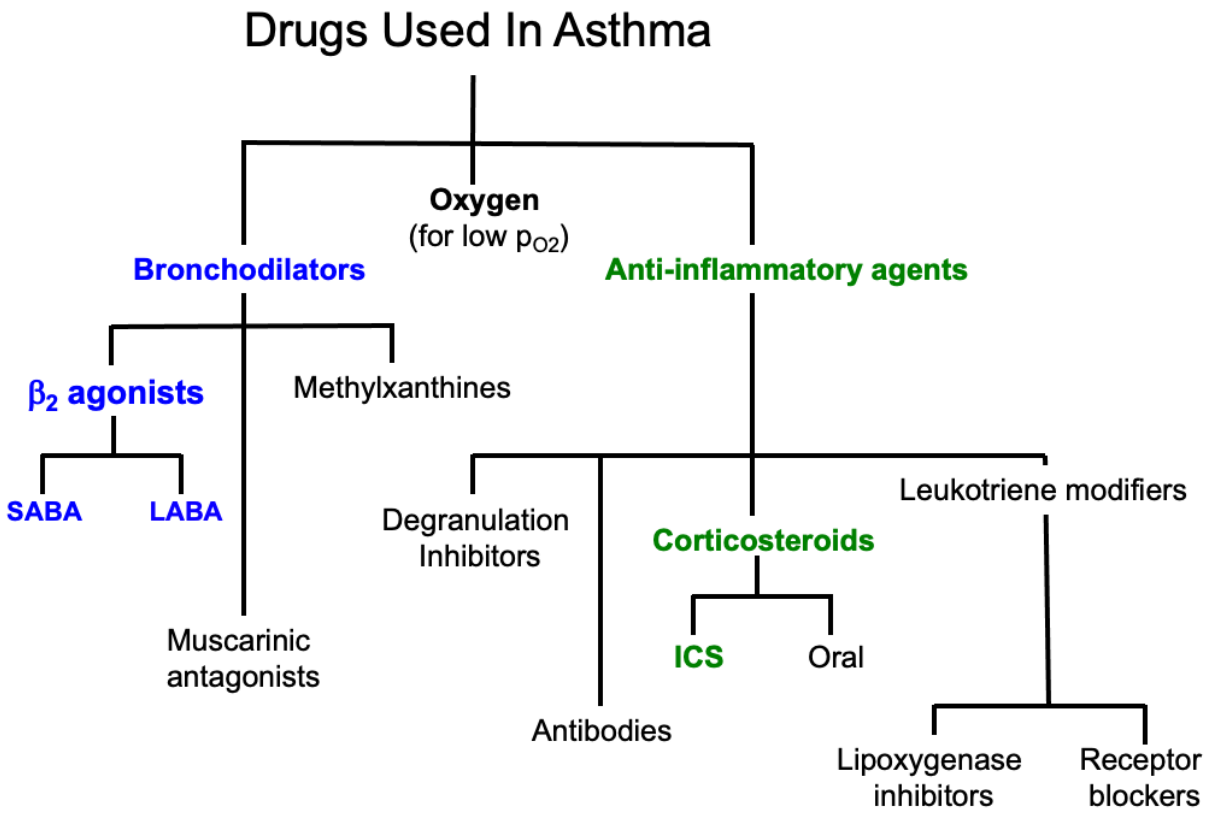



**Table 1: Classification of drugs used in asthma.**

## 2. AIM AND OBJECTIVES

### Aim:

The main aim of the present study is to evaluate the anti-asthmatic activity by using some standard procedures.

### Objectives:

●To select suitable animals for experimentation.

●To select the suitable standard drugs.

●Evaluation of anti –asthmatic activity of drugs using Histamine induced broncho constriction in anaesthetized guinea pig.

●Evaluation of anti –asthmatic activity of drugs using percent degranulation of rat mesenteric cells.

● Evaluation of anti –asthmatic activity of drugs using mast cell stabilization of mesentery of wristar rats.

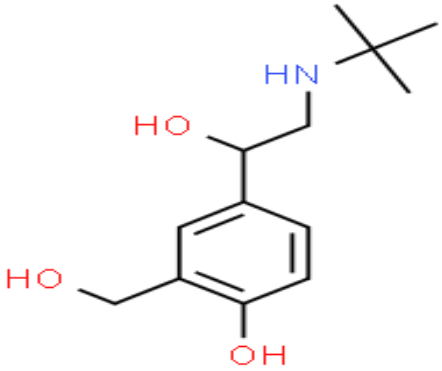
## 3. DRUG PROFILE

**SALBUTAMOL**

**Molecular formula**:C13H21NO3

**Molecular weight:**239.3107

**Chemical structure:**




**IUPAC Name:**4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hudroxymethyl)phenol

## Absorption:

Salbutamol acts topically on bronchial smooth muscle and the drug is intially undetectable in the blood. After 2 to 3 hrs low concentrations are seen.

## Volume of distirbution:

*The volume of distirbution recorded for intravenously administered salbutamol has been recorded as 156±38L.

## Protien binding:

- Weakly bound to plasma protiens.

## Metabolism:

Salbutamol is not metabolized in the lung but is converted in the liver to the 4'-o-sulphate (salbutamol 4'-o-sulphate) ester,which has negligible pharmacologic activity. It may also metabolized by oxidative deamination with glucornide.

## Route of elimination:

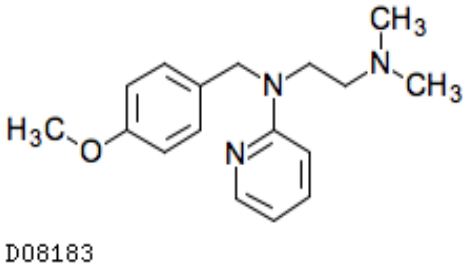
Salbutamol is excreted in the urine as free drug.

**MEPYRAMINE**

**Molecular formula**:C17H23N3O

**Molecular weight**:285.384

**Chemical structure:**

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**IUPAC Name**:N'-[(4-methoxyphenyl)methyl]-N,N-dimethyl-N'-pyridin-2-ylethane-1,2-diamine.

**Toxicity**:The acute over dosage of mepyaramine include convulsions,coma,ataxia.

●Mepyramine, is also known as pyrilamine, is a first generation anti histamine, targeting H1 receptor as an inverse agonist. It rapidly permeates the brain, often causing drowsiness.

●The medication has negligible anti cholinergic activity, with 130,000-fold selectivity for the histamine H1 receptors over the muscarinic acetyl choline receptors.

●It was patented in 1943 and came into medical use in 1949. It is used in over -the- counter combination.

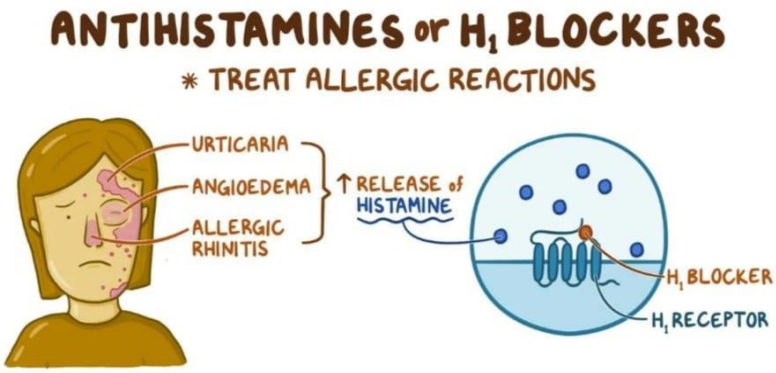


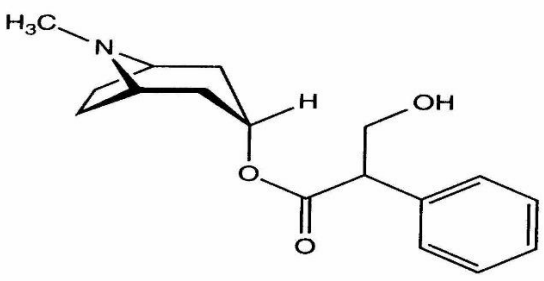

**Fig 3.1: Mechanism of $H_1$ Blockers.**

**ATROPINE SULPHATE**

**Molecular formula**:C17H23NO3

**Molecular weight**:289.3694

**Chemical structure:**




**IUPAC Name**: [(1*S*,5*R*)-8-methyl-8-azabicyclo [3.2.1] octan-3-yl] 3-hydroxy-2-phenylpropanoate; sulfuric acid

**Absorption:**Intravenous atropine sulphate follows a non linear pharmacokinetic model at doses between 0.5 and 4mg. In adults given 1.67mg of atropine intramuscularly, the $C_{max}$ was 9.6mg/ml and the T max went from 3 to 60 minutes.

**Volume of distirbution**: Atropine is distibuted throught the body. The total volume of distirbution of atropine ranged between 1.0 and 1.7L/Kg.

**Metabolism:** Atropine is mainly metabolized by enzyamatic hydrolysis in the liver. The major metabolities of atropine are noratropine,atropine n oxide tropine,tropic acid.

**Route of elimination**: Approximatey 13 to 50% of atropine is excreted in unchanged in the urine.In healthy volunteers given intravenous atropine ,29% of atropine was excreted in urine, along with 155 of an unidentified metabolite.

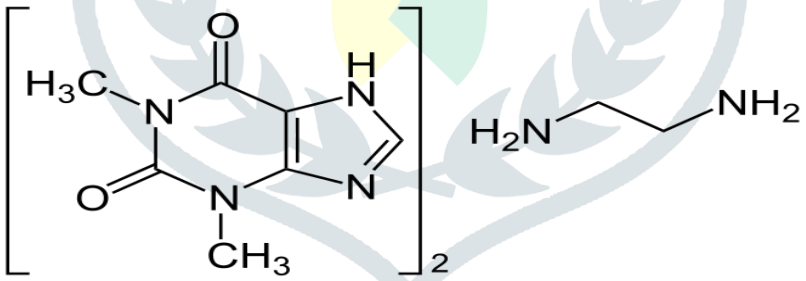
**Mechanism of atropine sulphate**: The most important therapeutic action of atropine is the inhibition of smooth muscle and glands innervated y postganglionic cholinergic nerves. It also has central nervous system activity, which may be stimulating or depressing depending upon the dose..

**AMINOPHYLLINE**

**Molecular formula**:C16H24N10O4

**Molecular weight**: 420.4264

**Chemical structure:**




**IUPAC Name**: 1,3-dimethyl-7*H*-purine-2,6-dione; ethane-1,2-diamine

**Volume of distirbution**: 0.3 to 0.7L/kg

**Protien binding**:60%

**Half life**:7-9 hours

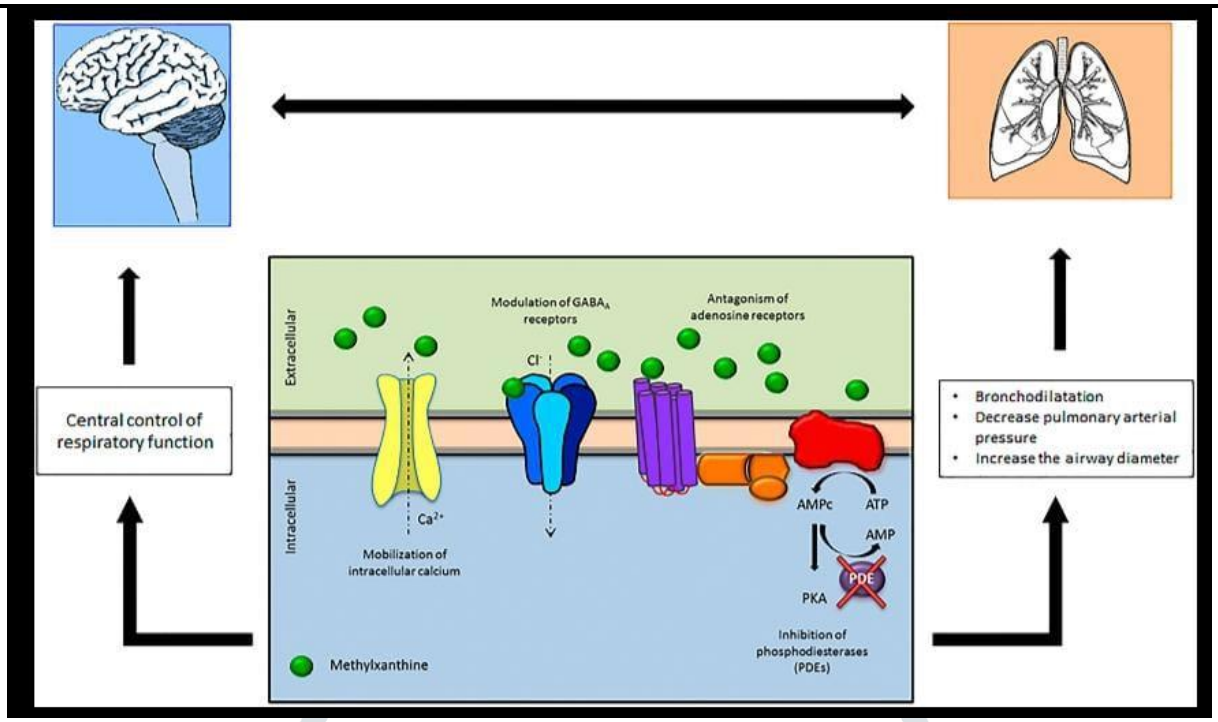


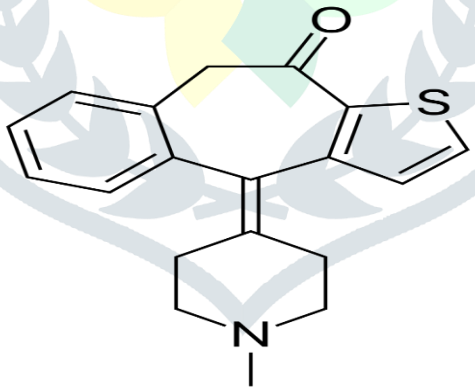

**Fig 3.2 Mechanism of aminophylline**

**KETOTIFEN**

**Molecular formula**: C19H19NOS

**Molecular weight**: 309.425

**Structure:**




**Absorption**: Oral administration, absorption is relatively quick (with a $_{Tmax}$of ã 3 hours)

**Protien binding**: 75% protein bound in plasma ,though the specific protiens to which it binds are nuclear.

**Route of elimination**:

More than 60% of an administerd dose is excreted in the urine, primarily as metabolites of this material, <1% is found as unchanged drug.

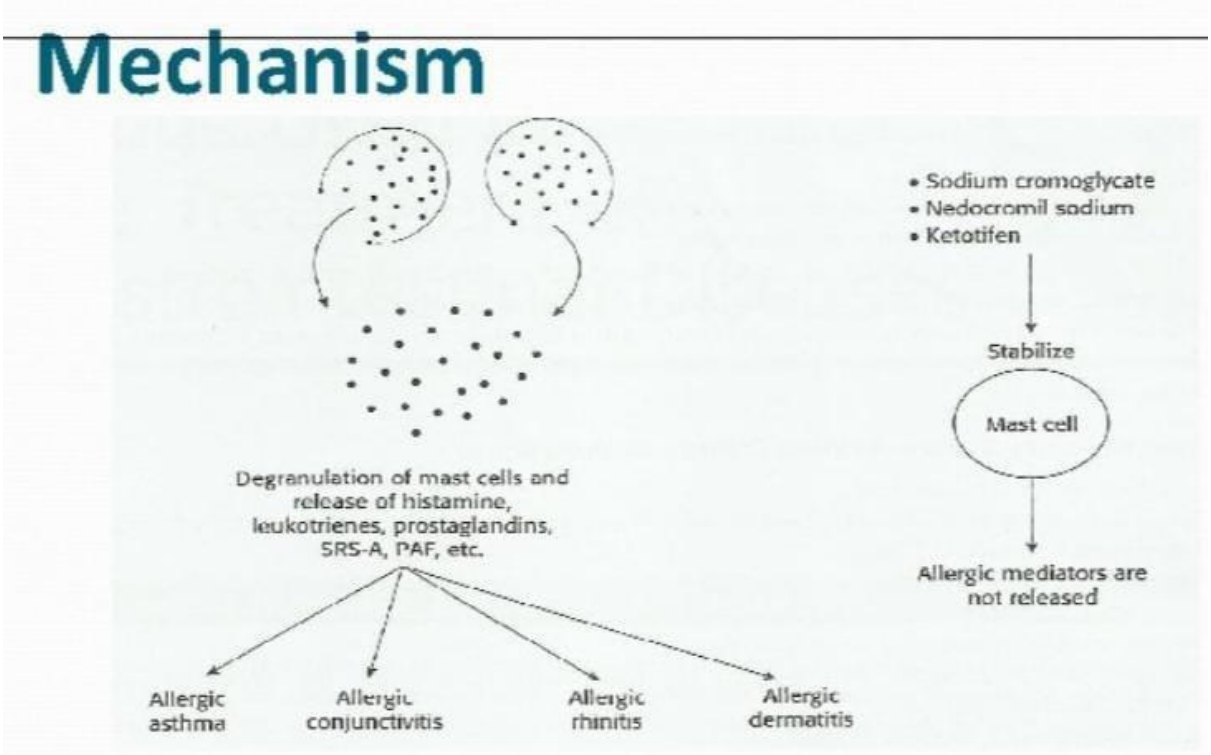


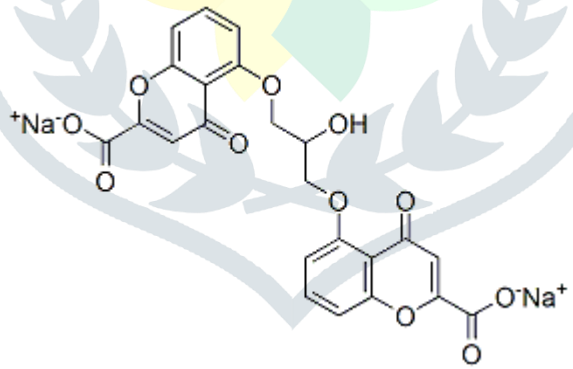

**Fig 3.3: Mechanism of ketotifin.**

**DISODIUMCROMOGLYCATE**

**Molecular formula**:C23H14Na2O11

**Molecular weight**: 512.334

**Chemical structure:**




**IUPAC Name:** Disodium 5- [3- (2-carboxylato-4-oxochr)]

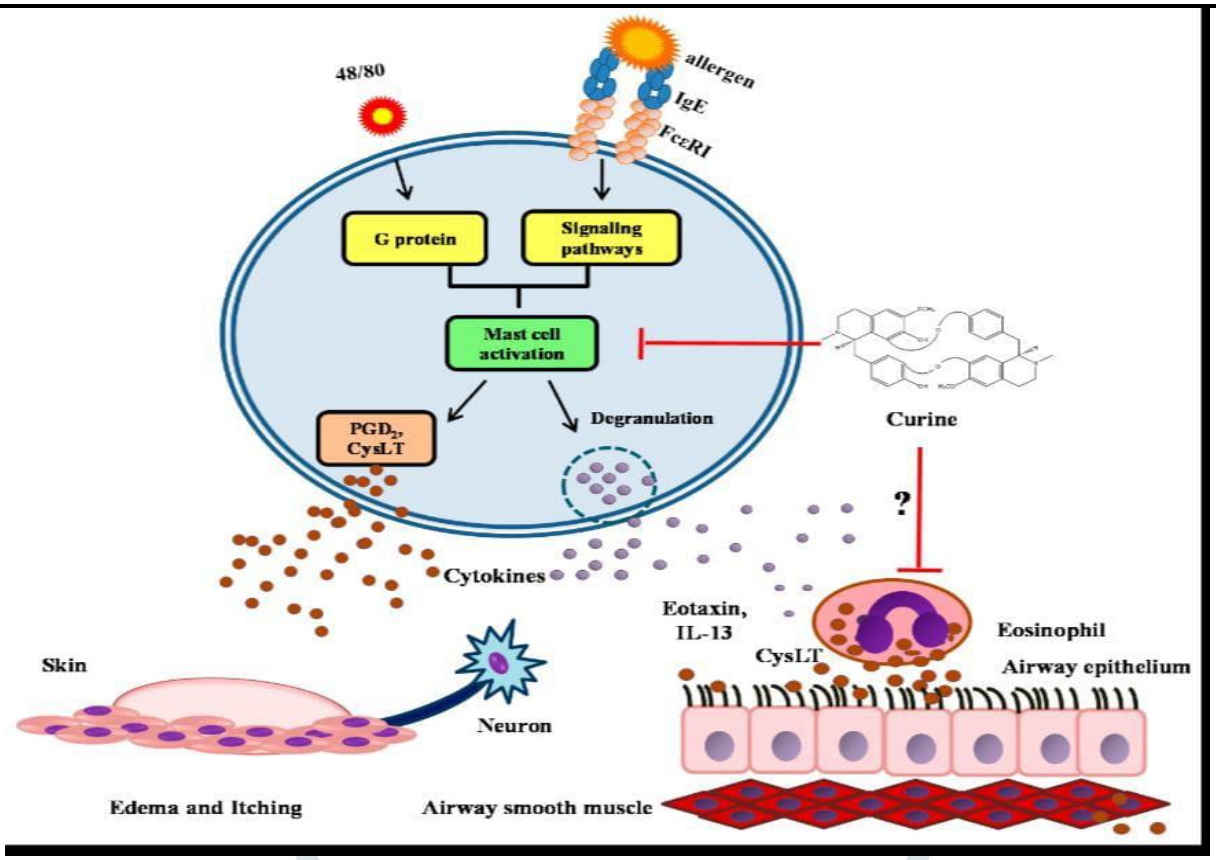



**Fig:3.4 Mechanism of disodiumcromoglycolate.**

## 4. MATERIALS AND METHODS

### 4.1 Materials:

**Drugs:**Salbutamol, Mepyramine, Atropine sulphate

**Chemicals used:**Pentobarbitone sodium

### Animals

Experiments were performed on Guinea pig of either sex. The animals were fasted for 24 hours.

### HISTAMINE- INDUCED BRONCHOCONSTRICTION IN ANAESTHETIZED GUINEA PIG:[12,13]

### *METHODOLOGY:

Guinea pigs of either sex weighing around 500g are anaesthetized with 70mg/kg of pentobarbitone sodium given intraperitoneally. The trachea,pleuralcavity,jugular vein and carotid artery are canulated.The animals are subjected to mechanical ventilation with a starling respiratory pump which delivers an inspiration volume presenting a tracheal pressure of 8cm of water at a rate of 60 strokes/min. A neuromuscular blocking agent like alcuronium(1mg/kg, s.c.)or succinyl chloride( 1mg/kg,I.v.) is given to prevent interface form spontaneous respiration . The animals are then placed inside a whole body plethismograph and tracheal,pleural,venous and arterial catheters are connected to onset ports in the wall of the plethysmograph box.The tracheal part is connected to the respiratory pump.Airflow rate into and out of plethismograph are measured as pressure difference using a differential pressure transducer. Airflow is caliberated by passing compressed air through a rotameter.The tidal volume (VT) is calculated from the flow signal.Transpulmonary pressure (PTP) is measured with a different measure transducer,with one side attached to a catheter inserted into the right pleural cavity and the other side

connected to a side port of tracheal cannula .PTP is caliberated with a water manometer.Signals from airflow,tidal volume and transpulmonary pressure are fed into an online computer system for calculation of pulmonary resistance (Rl) and dynamic lung compliance(Cdvn). Systemic arterial pressure is measured using a statham pressure transducer. Heart rate is computed from pressure pulses. Three doses of test compund (or) standard are injected intravenously. Control animals are injected saline. Intravenous injection of histamine (0.52-2mg/kg) lead to a short decrease in Cdvn and to short increase in Rl by approximately 200% compared with the base line. Challenges are repeated at 5 intervals, yielding the same increase in Rl during the whole 1H period of experimentation. After 3 reproducible responses, the test compund is administered intravenously 1 min before the histamine injection.

Inhibition of histamine induced bronchoconstriction by graded doses of test compound and standard is recorded, ED50 values for inhibition of Rl are calculated. The time courses of histamine antagonism is also evaluated. The whole body plethysmography technique has proven a very useful technique in respiratory pharmacology for studies on antagonism against various bronchoconstrictors like histamine,bradykinin,5-HT, leukotrienes etc. as well as for airway pharmacology of potassium channel openers.

## 2. Percent degranultion of rat mesentric cells:[14]

## *METHODOLOGY:

Degranulation of rat peritonial mast cells can be induced in in vitro by different stimuli egg albumin, sodium cromoglycolate and compund 48/80. This involves microscopic examination of rat mesentric cells mast cells.Adult male albino wistar rats were sacrificed and pieces of mesentry with connecting lobes of fat and blood vessels were rapidly dissescted out and placed in ringer locke solution. All the petri dishes were incubated for 30 minutes. The mesentries were transferred to other petridishes containing 0.1 ml of 1% w/v solution of egg albumin for 20 minutes seperately. Then all these mesentries were transferred in 4% formaldehyde containing 0.1% toulidine blue dye and kept a side for 20 minutes. After staining and fixation of mast cells, mesentric pieces were transferred through acetone and xylene two times and mounted on slides. Six pieces of each mesentry were used for each concentration of drug. Each piece was observed under high power light microscope.

## 3. Mast cell degranulation in mesentry of wistar rats:[15,16]

## *METHODOLOGY:

Female sprague dawley rats weighing between 120-150g are sensitized by intramuscular injection of 10 mg/kg of highly purified ovalbumin. Simlataneously 1ml of bordetella pertusis suspension (2×10 organism) is injected intraperitoneally. This leads to induction of IgE antibodies which get attached to the surface of mast cells and basophillic granulocytes. After 11 days the animals are challenged by intravenous injection of 25 mg/kg of highly purified albumin. This results in the formation of antigen-antibody complexes on the surfaces of mast cells and various mediators of anaphlyaxis such as histamine, 5-HT, and SRS -A etc. Leading to symptoms of severe shock and 80% mortality. Corticosteriods like dexamethasone (1-10 mg/kg s.c ) given 18h before challenge (or) disodium cromoglycolate (30 mg/kg ) before injection of ovalbumin are able to counteract the symptoms of shock. Test drugs are administerd prior to challenge by a suitable route and allowing enough time to produce their

pharmacological action. The symptoms of shock are scored and the percentage of mortality is recorded and compared with untreated controls.

## 5.RESULTS

## *HISTAMINE INDUCED BRONCHIOCONSTRICTION IN ANASTHETIZED GUNIEA PIG:

| Drug | Standard | % Increase in PCT |
|---|---|---|
| Salbutamol | 0.2mg/kg | 65.83 |
| Mepyramine | 8mg/kg | 58.5 |
| Atropine sulphate | 2mg/kg | 380 |

**Table 5.1:Effect of salbutamol,mepyaramine,atropine sulphate on anti asthmatic activity.**

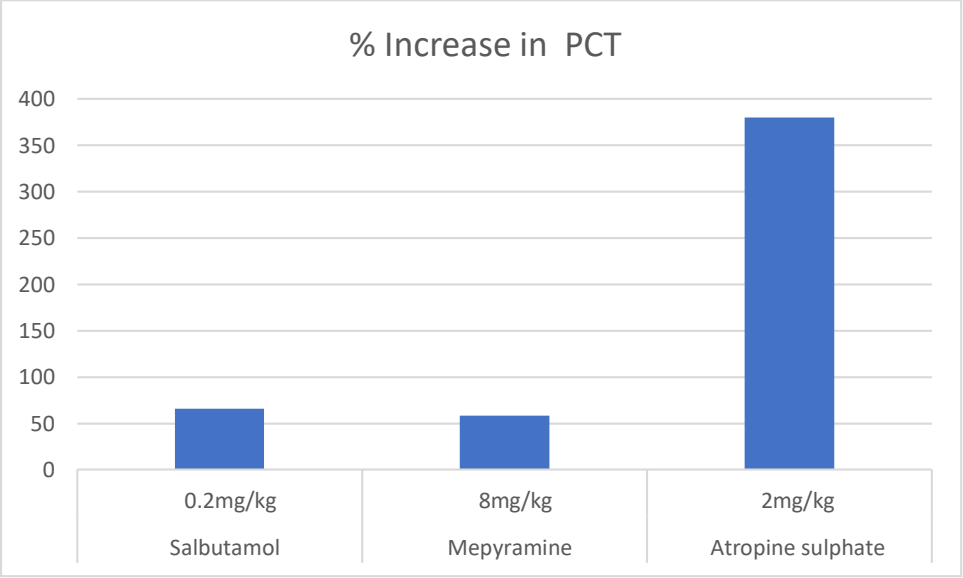



**Fig 5.1:Graphical representation of histamine induced broncho constriction in anaesthetized guniea pigs.**

## *PERCENT DEGRANULATION IN RAT MESENTRIC CELLS

| Drug | Standard | % Disrupted mast cells |
|---|---|---|
| Salbutamol | 0.2mg/kg | 56.5 |
| Aminophylline | 25mg/kg | 202 |
| Ketotifen | 1mg/kg | 87.35 |

**Table 5.2: Effect of salbutamol, aminophylline, ketotifen on anti asthmatic activity.**

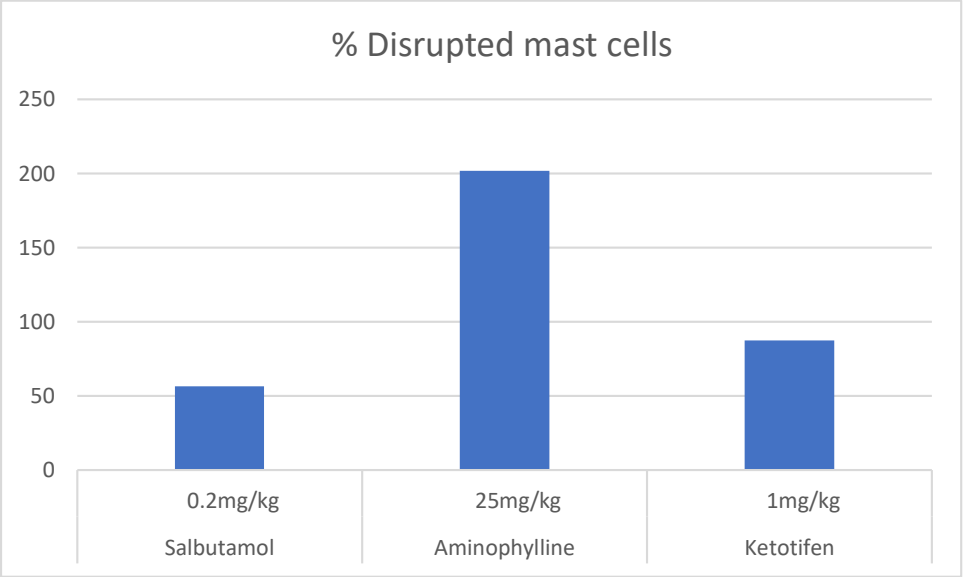



**Fig 5.2: Graphical representation of percent degranulation in rat mesenteric cells.**

## *MAST CELL DEGRANULATION IN MESENTERY OF WRISTER RATS:

| Drugs | Standard value | %Degranulation of mast cells |
|---|---|---|
| Disodium cromoglycate | 50mg/kg | 12.11% |
| Ketotifen | 10mg/ml | 21.93% |

**Table 5.3: Effect of disodium cromoglycate, ketotifen on anti asthmatic activity.**

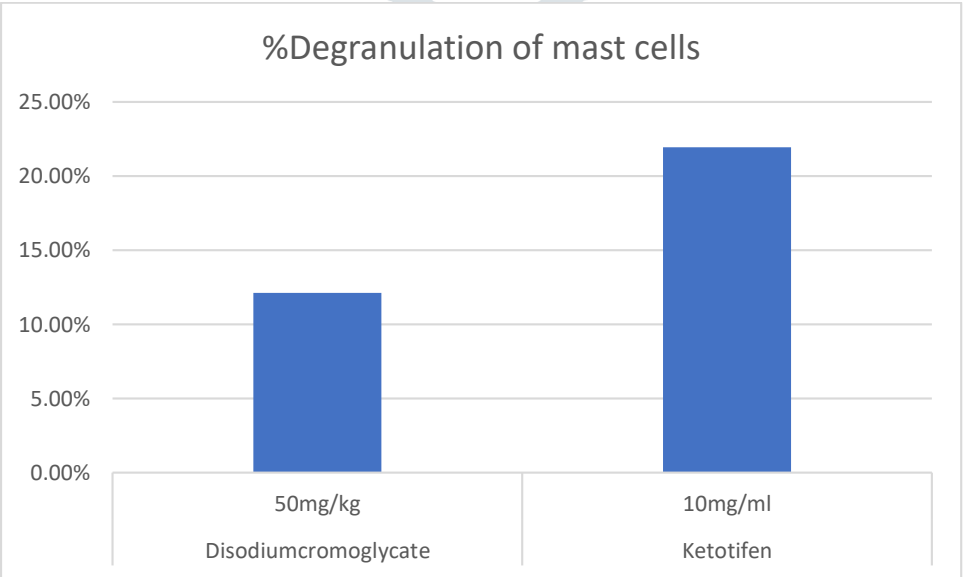



**Fig 5.3: Graphical representation of percent degranulation of mast cells.**

# DISCUSSION OF RESULTS

## HISTAMINE INDUCED BRONCHO CONSTRICTION IN ANASTHETISED GUNIEA PIG:

From the obtained result it was observed that there is a significant decrease in the asthma.

Atropine sulphate is administerd intravenously in histamine induce bronchoconstriction in anaesthetized guinea pigs.

The autonomic innervation of guinea pig air ways is also very similar to that of humans.The responses to experimental interventions in guniea pigs parallel the responses seen in human subjects. As with all animal models, guniea pig models of airways hyperresponsiveness are inadequate.But the key elements of airways reactivity functions similarly in humans and in guniea pigs.

### PERCENT DEGRANULATION OF MAST CELLS:

Mast cells play a key role in regulation of normal physiological process as well as in many pathophysiological settings.Histamine induces the production of proinflammatory cytokines, such as IL-6 and IL-8, and anti-athergonic ecosinoids. Considerable progress have been made in our understanding of cells in these recent years. Additional efforts to define the complex interactions of mast cells will potentially lead to novel clinical approaches for many pathologic condtion.

### MAST CELL DEGRANULATION IN MESENTRY OF WISTAR RATS:

In the present study, the role of histamine in modulating PMC degranulation was investigated. Our experiments showed as increase in degranulation of mast cells from infected animals after 15mins of histamine injection. The finding is consistent with previous studies reporting that changes in microvascular permeability produced by exogenous histamine are not just a direct action of this mediator on mesentric micro circulation, but also due to its effects on mast cell degranulation.

# SUMMARY AND CONCLUSION

The above studies and literature survey of screening procedures of anti-asthmatic activity of respective drugs show various anti-asthmatic activities and used to decrease respiratory problems like Cough, Wheezing.

*Atropine sulphate shows good anti-asthmatic activity than that of salbutamol, mepyramine.

*Aminophylline shows good anti-asthmatic activity than that of salbutamol,ketotifen.

*Ketotifen shows good anti-asthmatic activity than that of disodium cromoglycate.

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