

STUDIES ON BUTEA MONOSPERMA (PALAS) AS A POTENTIAL ANTI-ASTHMATIC AGENT

THESIS SUBMITTED
UNIVERSITY OF BOMBAY

FOR THE DEGREE OF
MASTER OF PHARMACY
IN
PHARMACOLOGY

SUBMITTED BY
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UNDER THE GUIDANCE OF
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**A
THESIS**

Submitted to
The University Of Bombay

For

The Degree Of
MASTER OF PHARMACY
Faculty Of Technology
In
PHARMACOLOGY

By

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..... UDAY R PHADKE.

PART I

INTRODUCTION AND PLAN OF WORK

INTRODUCTION :

Bronchial asthma is one of the oldest known diseases, which has got a history as old as human civilisation. In old Ayurvedic texts (2500 B.C.) this disease has been described. It also finds mention in the ancient Chinese medical books, believed to be written around 5000 B.C. It is a widespread malady affecting people from all walks of life and is seen in almost all parts of the world.

The word 'Asthma' was derived from the Greek word meaning 'difficulty in breathing', and this term was used loosely for centuries, to describe all types of shortness of breath. This created a lot of confusion until the first half of this century. But in last three decades, systematic attempts had been made to distinguish asthma from other airway obstruction diseases like emphysema, bronchitis, etc. by constructing a working definition of the disease, and its clinical features. This facilitated a choice of an appropriate drug therapy to combat the asthmatic attack, efficiently.

At present a number of drugs are at disposal of the clinician, either as preventive agents, or useful in symptomatic treatment (when an asthmatic attack is

on the way), but not a single of them is ideal as an antiasthmatic agent. An ideal antiasthmatic drug is one with a long bronchodilator action, inhibiting the mediator release caused by immune mechanism or otherwise and causing the desensitization of the patient against the specific allergen/s. The various mechanisms which are involved in the genesis of asthma, in different individuals or possibly in the same individual makes it very difficult to find a panacea. Hence, a single drug may not give complete relief and multi-drug therapy has to be continued.

It was estimated that almost 10% of the world population suffers or is likely to suffer from episodes of dyspnoea during its life-time (Pain, 1973). The magnitude of the problem emphasises the need for newer and better drugs to combat this condition. In this direction attempts were made in the laboratories, either from realms of synthetic chemistry or from the natural plant source.

The drugs used in present asthma therapy can be classified in four groups according to their mechanism of action.

1. Sympathomimetic amines,
2. Xanthine derivatives,

3) Corticosteroids,

4) Sodium chromoglycate and similar compounds.

To develop a new antiasthmatic agent, one way is to modify these known nuclei. The specific beta-2-receptor stimulant drugs like salbutamol, terbutaline are thus the modifications of the isoprenaline nucleus. With this approach, though the potency of the drug can be increased, the mode of action cannot be changed dramatically.

The other approach is to screen empirically, different unknown nuclei. This procedure is lengthy and expensive which may not yield any benefit at the end of the whole study and thus involves some risk.

Alternatively, exploitation of plant source may be done to develop new antiasthmatic agents. While selecting such plants as potential drugs, a bias may be given to the plants which are mentioned in Ayurvedic and other folklore medicines. A number of such plants and their extracts are described in Ayurveda either alone or in combination with other plants, to be effective as antiasthmatic drugs. If these plants are subjected to simultaneous phytochemical and pharmacological investigations, a pure active compound may come forth,

which has got a different nucleus than that of the established drugs and also a different mechanism of action. This compound may not be equipotent or more potent than the present drugs, but by modifying the new nuclei a more specific and potent drug can be developed. One good example of such isolation is vasicinone. The plant 'Adhatoda vasaka' has been mentioned in Ayurveda for the treatment of various bronchial diseases (Nadkarni, 1976). Amin and Mohta (1959) reported an alkaloid vasicinone with bronchodilator activity from this plant. Vasicinone is the oxidative product of another alkaloid vasicine which is also present in the plant. But this alkaloid possesses an exactly opposite action - bronchoconstriction and both these alkaloids are present in the unknown proportions in the extracts used in Ayurveda (Bhide et al., 1974; 1976). Hence, vasicinone alone will be preferable than the extracts which may contain unknown quantities of the two opposite acting alkaloids.

Disodium cromoglycate, which was developed by Cox et al. (1970) from active moiety Rhellin (Ami visnaga) is another example of exploitation of plant source.

The plant Butea monosperma, which has been described in Ayurveda to be of value in cough, cold and catarrh (Nadkarni, 1976), was selected with the aim to exploit the plant kingdom as potential anti-asthmatic agent source, instead of screening synthetic compounds. In the preliminary study methanolic extract of the bark showed a bronchodilator activity. This extract was used for further investigations. Partial purification of this extract was done to isolate an active fraction and pharmacological studies of this fraction were carried out with special reference to its anti-asthmatic and/or antiallergic activity and its mode of action.

PLAN OF WORK :

The work is presented in five parts :

Part - I : Introduction and Plan of Work.

Part - II: Literature Review.

Chapter-1 : Asthma Review :

1. Definition and Classification of Asthma.
2. Pathophysiology of Asthma.
3. Immunological Aspects of Asthma.

Chapter-2 : Treatment of Asthma :

1. Review of Present Treatment for Asthma.
2. Review of Plant Products as Antiasthmatics.

Chapter-3 : Review of Plant Butea monosperma.

Part - III: Material and Methods :

Chapter-1 : Phytochemistry :

1. Material.
2. Methods.

Chapter-2 : Pharmacology :

1. Material.
2. Methods:
 - a) in vitro.
 - b) in vivo .

Part - IV : Results :

Chapter-1 : Phytochemistry.

Chapter-2 : Pharmacology :

- a) in vitro
- b) in vivo

Part - V : Discussion and Conclusion.

PART II

LITERATURE REVIEW

Chapter - 1

ASTHMA REVIEW :

1. Definition and Classification of Asthma :

Bronchial asthma is a clinical state of heightened reactivity of the tracheobronchial tree to numerous stimuli. The airway obstruction caused in the disorder is observed as episodes of dyspnoea, Wheezing and cough with or without production of tenacious sputum. Bronchospasm, bronchial wall oedema, hypersecretion of mucus glands and collapse of small bronchioles are the root causes of these symptoms. The asthmatic attacks may be paroxysmal or continuous, the severity of which may range from mild to life threatening asphyxiation - status asthmaticus. However, these asthmatic attacks are reversible either spontaneously or through treatment (Weiss, 1975).

Definition :

It is very difficult to provide a satisfactory definition, inspite of a lot of work done to understand what asthma is. The superficial similarities of asthma with other bronchial diseases like emphysema and chronic bronchitis, makes it impossible to construct a universally acceptable definition. A convention of a study group was held under the auspices of the CIBA Foundation, in

1971, to identify and define asthma. It was agreed in the convention that asthma in itself is not a disease but symptoms of a disease (Scadding, 1971). The group referred the definition of asthma proposed by the 'CIBA Foundation Guest Symposium' (1958) as 'a condition of subjects with widespread narrowing of the bronchial airways, which changes its severity over short periods of time either spontaneously or under treatment and is not due to the cardiovascular diseases'. In the concluding remarks of the convention Fletcher et al. (1971) stated that this definition is not fully acceptable as it does not take into consideration the known types of asthma nor does it clearly mention the causes of asthma, and it is not possible, on the present information available to define asthma. However, the above definition has been accepted as the working definition, as it embraces all types of the conditions and stresses that the cause of the disease lies in the respiratory system only.

Many other workers in the field also have attempted to define asthma (Vaughan et al., 1972; Sherman, 1959; Howell, 1973; Spector, 1979). All these definitions are more or less similar to the above definition and cannot be accepted unanimously as complete definitions, but they also can be treated as working ^{definitions.} ~~conditions.~~

Classification :

In its classical forms asthma may be categorised in two groups, according to the characteristic features found in clinical situations. Though this classification is by no means entirely satisfactory it has been proved advantageous in selecting the proper therapy. The two important types are - 1) Extrinsic asthma, and 2) Intrinsic asthma.

Extrinsic asthma (Allergic, atopic asthma) is characterised by attacks of asthma provoked by allergens, giving flare and wheal type hypersensitivity and most of the times is mediated by reaginic IgE (Immunoglobulin E) type antibody. It usually affects children and adults below 25-30 years. On exposure to the specific allergen/s the patient shows characteristic bronchospasm paroxysmally with wheezing, dyspnoea and other respiratory distresses. The attacks are sudden with brief durations and most of the symptoms disappear between the periods of such attacks. The patients often have a history of other allergic manifestations as hay fever, eczema, etc. and have a family history of such atopy. The treatment usually produces a favourable response, but recurrence may be observed in adult life in a number of patients.

Intrinsic (Psychogenic, nonatopic) asthma is usually seen perenially and develops in adult after about the age of 35. It is recognisable against the extrinsic asthma as allergic factors hardly play any apparent role in etiology of this type and infective agents may be considered as the responsible factors. Hence, this is also known as idiopathic or infective asthma. However, an occasional history of atopy is also seen. The treatment may not relieve the symptoms completely and the condition may become chronic with development of cough and production of sputum in the intervals between acute attacks.

The episodes of both types of asthma are indistinguishable, except the sputum production but cough is severe in intrinsic type and to differentiate the correct type of asthma, a complete history of patient (like age, presence of infection in tracheo-bronchial tree, skin tests, etc.) must be available

Miscellaneous types : In addition to these two types, some cases of asthma due to reaction between allergens and nonreaginic antibodies in nonatopic subjects are seen and these are called as 'extrinsic nonatopic asthma'. Some times a combination of allergic and infective asthma may also be seen in some patients

called as 'Mixed type asthma'. 'Status Asthmaticus' is another subtype, which is a medical emergency, with wheezing, dyspnoea and other respiratory distresses symptoms that are refractory to the usual therapy. This also may prove fatal if not treated promptly and adequately.

2. Pathophysiology of Asthma :

Innervation of Tracheobronchial Tree :

The trachea, bronchi and lungs are innervated by autonomic nervous system (autonomic afferents, parasympathetic efferents, and sympathetic efferent pathways) through vagus nerves, pulmonary plexus and thoracic ganglia. The bronchial smooth muscle tone (which is mainly responsible for narrowing of airways), is maintained by vagal (cholinergic) and sympathetic (adrennergic) innervations.

Normal and Asthmatic Response of Tracheobronchial Tree :

The normal air breathed contains three million foreign particles per cubic feet, like dust, pollen, microorganisms, etc. ranging in size from $0.1\ \mu\text{m}$ to $1/2\ \text{mm}$. Depending upon their size the particles reach at different sites in the airways. A number of physiological and anatomical protective mechanisms are present,

like the mucous secretion, ciliary movement, phagocytosis, lymphatic drainage and the immunological mechanisms to eliminate these harmful particles. In normal system these mechanisms do not impair the normal respiratory function and ensure that air reaching to the alveoli is warm, moist and free of foreign particles. But in asthmatic subjects these mechanisms are exaggerated to give a response which may itself cause untoward effects like dyspnoea, hypersecretion, mucosal oedema, etc.

Pathophysiological changes in an Asthmatic Attack :

Pathological changes : The gross histopathological changes in the tracheobronchial tree during attacks or after death due to asthma are same regardless the cause of attack. These changes can be broadly stated as :-

- 1) Hypertrophy of smooth muscle with increase in size and number of muscles.
- 2) Intense infiltration of the oedematous interstitial tissues with eosinophils.
- 3) Hypertrophy of mucus glands and goblet cells.
- 4) Thickened and hyalinised basement membrane.

5) Shedding of epithelium causing interruption of continuous lining.

6) Reduction in bronchial lumen by eosinophils, epithelial cells, secretions and debris.

The net result of these pathological changes is overdistension of lungs with regional parenchymal zones of hyperinflation alternating with areas of atelectasis caused by thick, tenacious, intraluminal mucous plugs in bronchi and bronchioles. Reduction in luminal diameter is also seen due to smooth muscle contraction. These changes are seen depending upon the severity of attacks and are usually reversible. The almost normal features of the lungs are restored as the attack subsides.

Physiological changes : The characteristic physiological changes during an asthmatic attack are :-

1) The segmental and smaller bronchi are constricted (tending to complete closure) causing a reduction in vital capacity (VC) and dyspnoea. Even a mild attack causes a reduction in VC.

2) Increase in transpulmonary pressure (to counteract constriction), and distending pressure of bronchi. Hence the patient breathes at total lung capacity (TLC).

3) Bronchi, though assume their normal size, they are more susceptible to collapse at expiration than in normal subjects.

4) Breathing much nearer to total lung capacity is the principle cause of early metabolic and circulatory changes as increased oxygen consumption, increased cardiac output and pulmonary hypersecretion.

5) The changes are different in different parts of the lungs which may cause underventilation of certain areas, causing decrease in arterial oxygen tension (PO_2). Usually, the arterial carbon dioxide tension (P_{CO_2}) remains unaltered by a maintained total ventilation.

Immunological Aspects of Asthma :

Helmont in 17th century prophetically guessed the presence of a 'poyson', as the cause of the disease, in his definition of asthma. According to Donald (1971), this poison was nothing but the allorgens involved. Cooke and Vander Veer (1916) after studying 621 asthmatic patients, described the human protein sensitisation due to exposure to animals, pollens, etc., 48% of which showed a familial history. Atopy (in Greek unusual or strange) was the word suggested by Coca and Cooke (1923) for such a condition. Coca and Grovo (1925) described specifically reacting substances in the serum of atopic

individuals and called them atopic reagin. The reaginic antibody was called as Immunoglobulin E (IgE) first by Ishizaka et al. (1966) following its identification and characterization. This was supported by Johansson et al. (1968) and World Health Organization Report (1968). The identification of IgE protein as a carrier of reaginic activity provided several approaches to the study of reaginic hypersensitivity.

In a normal subject, exposed to a foreign matter, different immunologically nonspecific (as physical barriers, mucous, chemical, etc.) and specific protective mechanisms are stimulated. Immunologically specific mechanisms involve secretion of immunoglobulins from mucosa to act against foreign antigens. The specific immunoglobulins (antibodies) like IgE, IgG, IgM, IgA and IgD are produced to specific antigens (allergens) depending upon site of penetration of antigen. These antibodies form complex with antigen and this complex is eliminated by phagocytosis, destruction, etc.

But some times this normal response is exaggerated which gives rise to a secondary response due to which abnormality in normal functioning is seen. Four such types of reactions are recognised in man and are classified as Type I, II, III and IV reactions.

These reactions cause different diseases. These are summarised in Table-1 (Weiss, 1975).

The IgE antibody is responsible for the immediate hypersensitivity reaction (Type I) in atopic subject (Ishizaka and Ishizaka, 1970). This reaction provokes an immediate attack of asthma (15-30 mins.).

The IgG antibody is involved in the type III reaction (Hargreave and Peppys, 1972), complement system playing a major role in the reaction. A late asthmatic attack (within 6-8 hrs.) is precipitated by this reaction.

Dual type I and type III asthmatic reactions may also take place, e.g., due to chemicals (in occupational asthma).

The involvement of type II reaction in pathogenesis and immunological mechanisms of asthma is not yet known except in a few intrinsic asthmatic subjects.

The type IV reaction is not involved in asthma.

Thus, type I reaction is mainly responsible for extrinsic atopic asthma, while type III is important in extrinsic nonatopic or intrinsic asthma (Peppys, 1971).

Table - 1 : IMMUNE REACTIONS

TYPE OF REACTION	Time of Reaction	Example of Antigen	Antibody or Cell	Disease
I (Immediate hyper-sensitivity)	15-30 min.	Pollens, food.	IgE	Extrinsic asthma, Anaphylaxis, Hay fever.
II (Cytotoxic response)	--	Cell membrane, basement membrane	IgG, IgM ($\pm$ complement)	Hemolytic anaemia.
III (Arthus phenomena) Anti-G, anti-D complex	6-8 hrs.	Moulds, dust, etc.	IgG (complement)	Allergic alveolitis.
IV (Delayed hyper-sensitivity)	48 hrs.	Tubercular protein	Sensitized lymphocytes, monocytes.	Tuberculosis.

Immunoglobulins :

So far five types of immunoglobulins are identified - IgA, IgM, IgG, IgE and IgD. The molecular structure of all these classes is basically similar (Cohen and Porter, 1964) as shown in Figure-1.

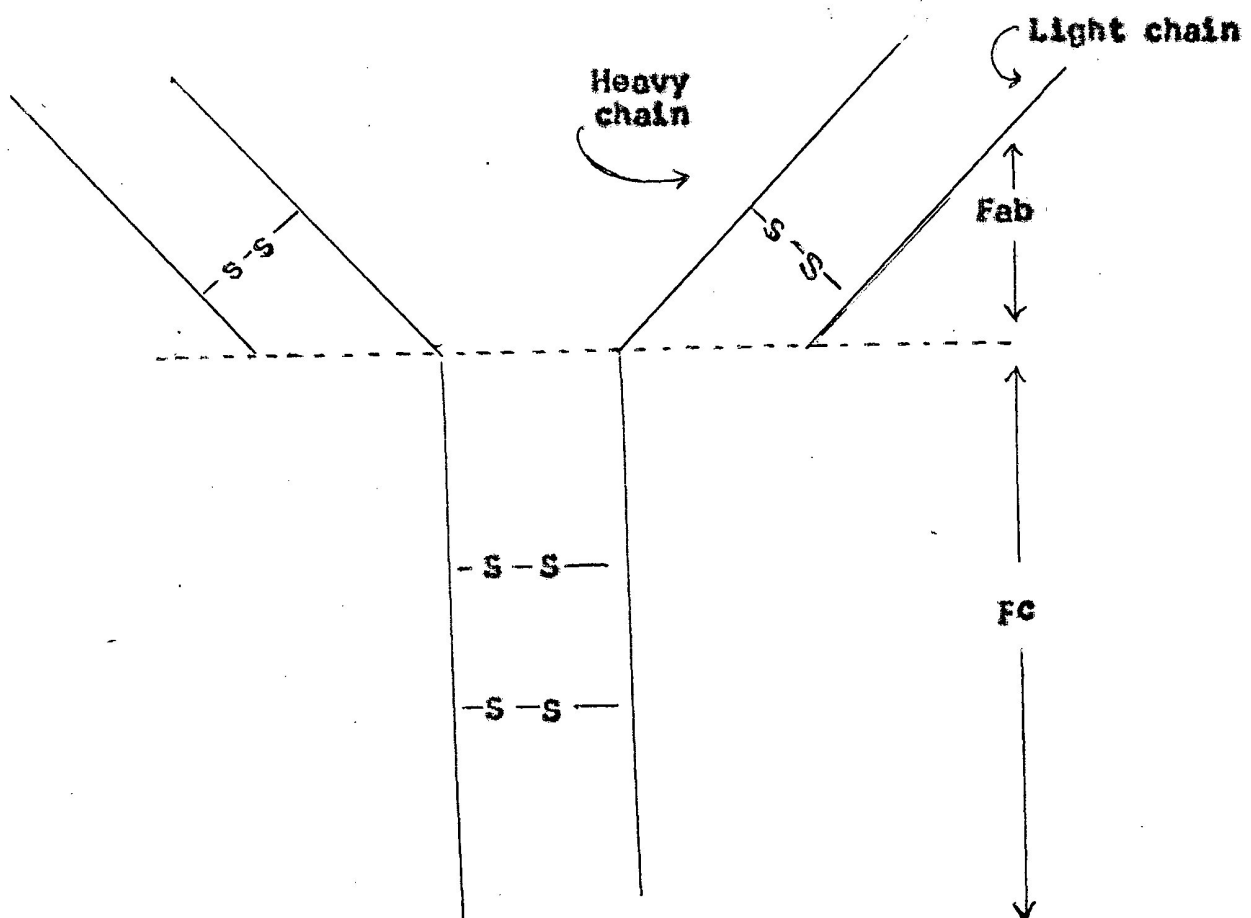


Fig. 1

Each monomer is composed of two long (heavy) and two short (light) peptide chains linked by a number of disulfide bonds. The amino acid sequence of the Fc

(fragment crystallisable) fraction of the heavy chains is characteristic for the particular class of antibody. The light chain sequence is similar for all types. Fc fraction determines the biological properties (ability to adhere cell surfaces, interaction with complement) while the Fab (Fragment antibody binding) component is responsible for specific antigen.

Immunoglobulin E

Molecular weight 187,000

Fc fraction fixes to receptor sites on mast cells and basophils and is heat labile (Ishizaka et al., 1967). It has short half life of 2-3 days in serum, but 9-14 days in tissue. It is formed in lymphoid tissue and plasma cells of the upper respiratory tract, tonsils, trachea and main bronchi (Ishizaka and Ishizaka, 1970). The levels of IgE in atopic subjects in nasal secretions are raised (Hobday et al., 1971). These levels are far greater than in non-atopic individuals in whom, IgA is mainly involved as defence mechanism.

Common allergens involved in type I reactions :

House dust, pollens, moulds, food allergens, danders, bacterial allergens, drugs (aspirin, penicillin).

Immune reaction (in immediate hypersensitivity):

In an immune reaction a sequence of events take place, in which two distinct phases are seen. The first

is the sensitisation phase and second, the challenge phase.

In sensitisation phase the atopic subject is exposed to a specific allergen. The plasma cells of the respiratory mucosa respond to this by forming specific antibodies of IgE class. These IgE antibodies attach themselves to the mast cells and basophil surface.

In the second phase, a re-exposure to the same allergen results in a challenge reaction. The antigen reaches the cell bound antibody (IgE) and the antigen-antibody reaction takes place which triggers a series of events involving Hageman factor, the complement system, phospholipases and the lipid endoperoxidase. As a net result of this reaction a number of substances are formed and/or liberated, which are known as mediators of anaphylaxis. The net sum effect of the mediators is smooth muscle contraction, vasodilatation and mucus hypersecretion that results in typical asthmatic attack.

Pharmacological mediators :

The list of mediators liberated during anaphylaxis (Middleton, 1980) include :- 1) Histamine, 2) Slow reacting substance of anaphylaxis (SRS-A), 3) Prostaglandins, 4) Bradykinin, 5) 5-Hydroxytryptamine, 6) Eosinophil chemotactic factor of anaphylaxis (ECFA).

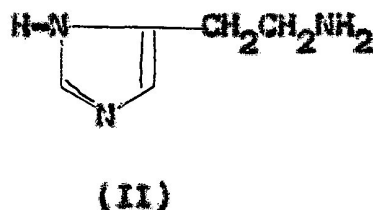
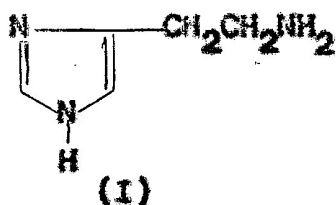
Heparin, platelet activating factor, chymase, lipid chemotactic factor, superoxide radicle are also liberated .

Histamine (β -aminoethyl imidazole) :

History (Greek: Histos-tissue) : Histamine was first synthesized by Windaus and Vogt as long back as 1907. Its existence in nature was later demonstrated by Barger and Dale (1911) by isolating it from ergot and intestinal mucosa. Its different pharmacological actions (like smooth muscle stimulation, vasodilation, etc.) was studied by Dale and Laidlaw (1910, 1911). These actions resembled with that of many tissue extracts. The similarity of high doses of histamine with reaction to foreign proteins, opened a new field of study of histamine release in anaphylactic reactions. Since then a lot of work has been done to establish histamine as the most important mediator, released in antigen-antibody reactions.

Chemistry, Biosynthesis and Degradation :

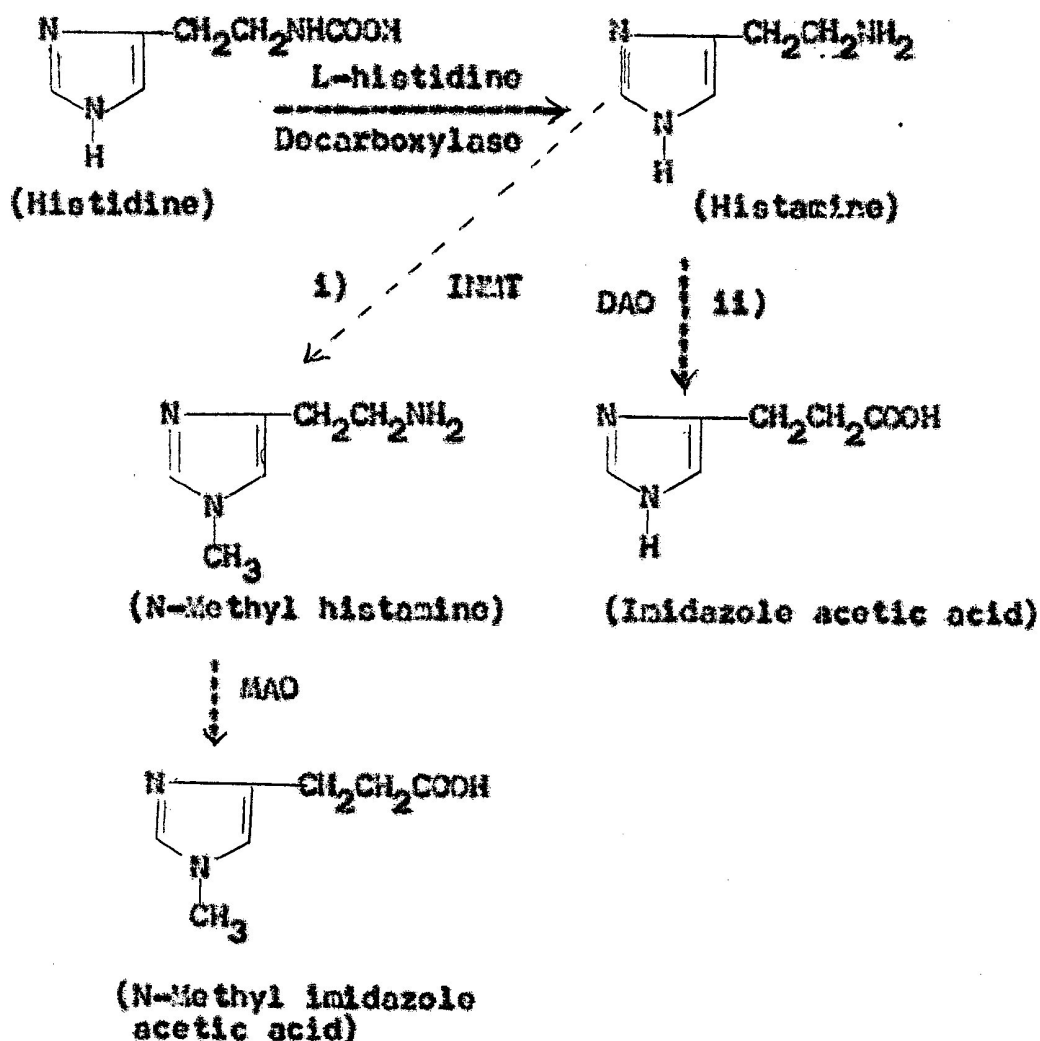
Histamine appears in two tautomeric forms.



It is biosynthesised in tissues by decarboxylation of histidine in presence of L-histidine decarboxylase.

Degradation occurs by two major pathways -

(i) ring methylation catalysed by histamine N-methyl transferase (HNMT) to N-methyl histamine most of which is converted to N-methyl imidazole acetic acid by monoamine oxidase (MAO); (ii) oxidative deamination by diamine oxidase (DAO) to give imidazole acetic acid.



Storage and Release :

Histamine is stored in mast cells in tissue or basophils in blood (Riley and West, 1966), in the form of a complex with heparin and protein (Chakrin and Krell, 1980), as secretory granules. It is synthesized in the mast cell or in other non-mast cells as human epidermis, etc. (Hakanson et al., 1967). The mast cell histamine turnover is slow while that of non-mast cell is fast.

Histamine in body is released by different pathways :-

1) Physical :

- a) Injury, stress induced release;
- b) Heat, mechanical release.

2) Chemical :

- a) Drugs, peptides, venoms induced release, detergents, bile salts;
- b) Histamine liberators (like compound 48/80 induced release).

3) Physiological release :

- a) In growing and regenerating tissue.
- b) In CNS as a mediator.

4) Pathological release :

- a) In gastric ulcers,
- b) In anaphylactic and allergic reactions.

Of all these mechanisms, histamine release in anaphylactic reactions is most important in the present context.

When a specific antigen combines with a mast cell bound antibody (IgE) the process of histamine release is initiated (Ishizaka and Ishizaka, 1978). This involves calcium ions and metabolic energy (Ennis et al., 1980). The antigen-antibody complex increases the permeability of cell membrane, causing influx of Ca^{++} ions, which may inhibit the adenylyl cyclase, stimulate guanylyl cyclase or control the phosphodiesterase regulatory protein. This causes a decrease in cyclic adenosine monophosphate (cAMP) or increase in cyclic guanosine monophosphate (cGMP) which induces the release (Diamant et al., 1978). Thus Ca^{++} ions are necessary for the process of degranulation and the release of histamine by exocytosis (Douglas, 1968). The involvement of Ca^{++} ions in the process has been proved by many workers with

I) Stimulating histamine release by

- i) Microiontophoresis of Ca ions in mast cells;
- ii) Using Ca ionophore like A-23187 in rat mast cells (Foreman et al., 1973), human mast cells (Behrendt et al., 1978), or X-537 A in rat mast cells (Diamant et al., 1978).

II) Inhibiting the release by

- 1) Removal of extracellular Ca (Monger and Schild, 1958);
- ii) Addition of Ca antagonist as quercetin (Payne, 1978).

Pharmacological effects in Anaphylaxis :

Histamine release increases the capillary permeability and causes vasodilation, which causes oedema. The mucus secretion is also increased. It causes bronchoconstriction in vivo by contracting bronchial smooth muscle (Nadel, 1973). In vitro or in vivo histamine release in anaphylactic reactions can be demonstrated in active or passive anaphylaxis in different tissues as lung, peritoneum, etc.

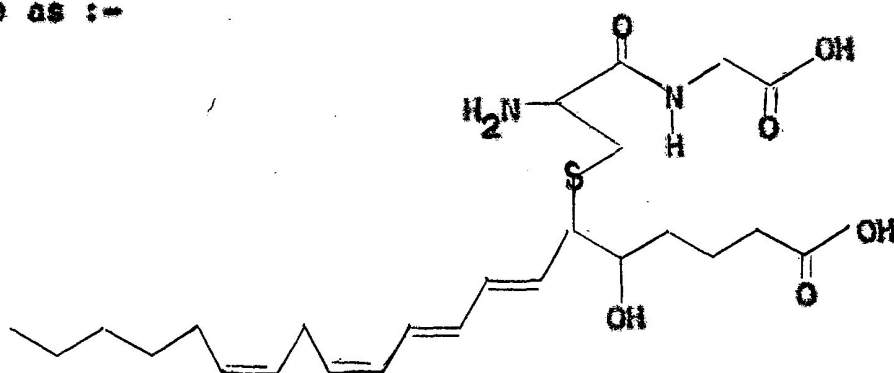
Slow reacting substance of Anaphylaxis (SRS-A):

History : SRS-A is one of the primary mediators formed and released during anaphylactic reactions and plays a major role in bronchoconstriction in human asthma. Its release was initially detected in isolated perfused lungs of guinea pig (Kellaway and Trethwaite, 1940; Brocklehurst, 1960) and subsequently, in vivo, in guinea pigs (Collier and James, 1967; Stechschulte et al., 1973). In sensitised human lung, in vitro, SRS-A release by challenge with the specific antigen was shown and this

SRS-A was found to be similar to guinea pig SRS-A (Sheard *et al.*, 1967). Brocklehurst (1960), termed the slow reacting substance released immunologically as SRS-A, to differentiate it from non-immunological SRS released by other agents like snake venom.

Chemistry, Biosynthesis and Degradation :

Chemical structure of SRS-A had remained unknown for a long time. Samuelson and co-workers (1979) demonstrated that the structure is not of any known chemical nucleus and labelled this group as leukotrienes. Morris *et al.* (1980) later elucidated the structure as :-



SRS-A

It is synthesized in body from arachidonic acid (Jackshick *et al.*, 1977), and shares the basic eicosatetraenoic acid structure of arachidonic acid.

Storage and Release :

SRS-A is not stored in a preformed state in cells but is synthesized and released during anaphylaxis (Austen, 1973) or by challenge with calcium ionophore A 23187 (Bach and Brashler, 1974). Previously it was believed that mast cells are responsible for its synthesis and release. The recent studies showed that polymorphonuclear leucocytes may be the more important cell types for optimal release of the mediator (Burka and Flower, 1979). The mechanism of action of this release is very much similar to that of histamine and it involves the Ca^{++} ions.

Pharmacological effects in Anaphylaxis :

SRS-A contracts human isolated bronchial smooth muscle and guinea pig isolated trachea, partially purified SRS-A causes bronchoconstriction in guinea pigs in vivo (Brocklehurst, 1960; Berry and Collier, 1964). Administration of SRS-A by aerosol to asthmatic patients causes broncho constriction (Herxheimer and Stressman, 1963). It increases the vascular permeability of the skin, and this might be the cause in lungs for oedema formation and obstruction of airways.

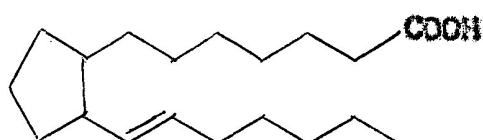
Prostaglandins :

History : Kurzork and Lieb (1930) showed the relaxation or contraction of human uterus with human

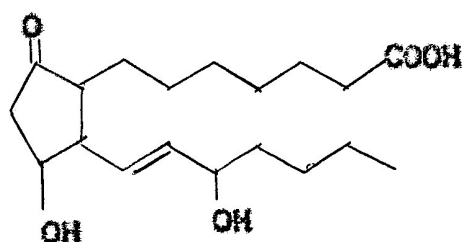
semen. Goldblatt (1935) and Euler (1936) then showed the muscle contracting and vasodepressor activity of seminal fluid and other reproductive glands. Euler (1936) designated this active substance as prostaglandin. Next, it was found that prostaglandins were a family of compounds of unique structure. PGE , PGE_2 , PGF_1 , PGG_2 , etc. are some of the important prostaglandins. Prostaglandins were found to be released during anaphylactic reactions by Piper and Vane (1969); Piper and Walker (1973).

Chemistry, Biosynthesis and Degradation :

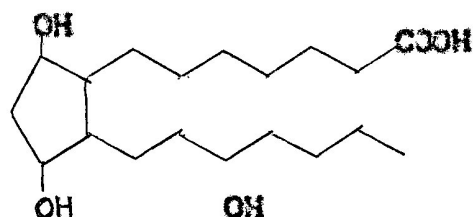
Prostaglandins are essential fatty acids. They are considered as analogues of an unnatural compound prostanoic acid which is a twenty carbon compound.



(Prostanoic acid)



(Prostaglandin E)



(Prostaglandin F_1)

They fall into several main classes, designated by letters and distinguished by substitution on cyclopentane rings.

They are biosynthesised from arachidonic acid as its metabolites, by cyclo-oxygenase enzyme (particularly primary prostaglandins).

Degradation involves two steps :-

1) Oxidation of 15-OH group to ketone by prostaglandin 15-OH dehydrogenase (Hamberg and Samuelson, 1971).

2) Reduction of 15-keto compound to 13,14-dihydro-derivative by prostaglandin Δ^13 reductase (Anggard et al., 1971).

Storage and release :

Prostaglandins are released from lung tissue by a variety of stimuli including anaphylaxis. They are not preformed but synthesized and released after the stimulation (Piper and Vane, 1971). Prostaglandins are probably formed by virtually every tissue and cell type (Douglas, 1980).

Pharmacological Effects in Anaphylaxis :

Prostaglandins contract (PGFs) or relax (PGEs) bronchial smooth muscles. An abnormal imbalance of PGF₂ over PGE₂ could contribute to high bronchial tone in asthma.

Bradykinin (Greek - Bradye-slow, Kincin - to move) :

History : The presence of bradykinin in the blood of anaphylactic dog was reported by Beraldo (1950). Brocklehurst and Lahiri (1962) suggested that bradykinin produced during anaphylaxis in guinea pigs, rabbits and rats, contribute to anaphylactic syndrome. Elliot et al. (1960) purified bradykinin by action of crystalline trypsin on pseudoglobulin of ox blood. Boissennas et al. (1960), achieved its chemical synthesis.

Chemistry, Biosynthesis and Degradation :

Bradykinin is a nonapeptide, formed by action of enzyme kallikrein on the precursor kininogen, in the plasma γ_2 -globulin fraction. The amino acid sequence of bradykinin is Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg.

Bradykinin is metabolised by two principal kininases :- 1) Kininase I remove C terminal arginine; 2) kininase II remove C terminal dipeptide, both yielding virtually inactive peptides (Erdos, 1976).

Storage and Release :

It is not stored in the preformed state in body but is released in the anaphylactic shocks. Kinninogen, the precursor of bradykinin is found throughout the body.

Bradykinin is formed in tissue mainly and to a less extent in blood (Brocklehurst and Lahiri, 1962).

Pharmacological Effects in Anaphylaxis :

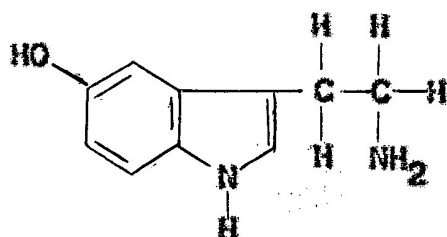
Bradykinin causes bronchoconstriction in guinea pigs (Collier and James, 1966), but dilatation as well as constriction in other species. Inhalation of kinins may lead to a respiratory distress in asthmatics (Newball *et al.*, 1975). Bradykinin in guinea pigs *in vivo* causes release of rabbit aorta contracting substance (RCS) (Palmer *et al.*, 1973) and prostaglandin E (Juan, 1977).

5-Hydroxy tryptamine (5-HT, Serotonin):

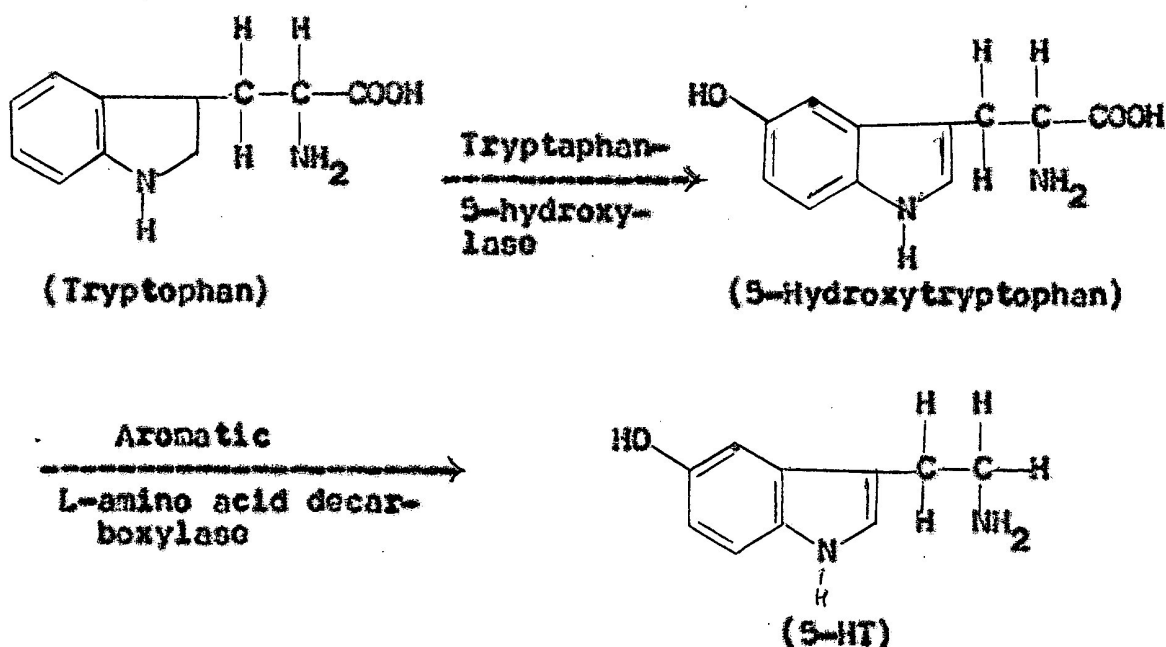
The vasoconstrictor property and occurrence in the serum of clotted blood of 5-HT was first recognised by Ludwig and Schmidt in 1868, though its chemistry was not known. It took almost 80 years to isolate (Rapport *et al.*, 1948) and identify (Rapport, 1949) this substance as 5-hydroxy tryptamine. Hamlin and Fischer (1951) prepared it synthetically with all properties of natural one..

Chemistry and Biosynthesis :

It is a 3-(β -aminooethyl)-5-hydroxy indole.



It is formed enzymatically from the amino acid tryptophan by hydroxylation and subsequent decarboxylation as :-



Storage and Release:

5-HT is synthesized and stored in enterochromaffin cells, neurones and mast cells, while platelets take it up from external tissue. It is stored in cells as a non-diffusible complex with ATP and divalent cations, enclosed in vesicles.

Release of 5-HT in antigen-antibody reaction in vitro was first demonstrated by Humphrey and Jacques

(1955). West (1958) demonstrated its release in various species. Chasin et al. (1979) described its release from rat peritoneal cells.

Pharmacological effects in Anaphylaxis :

5-HT causes bronchoconstriction in many animals, including man and particularly the asthmatics. The effect is partly reflex but mainly due to direct stimulation of bronchial smooth muscle (Radbard and Kiro, (1972).

Eosinophil chemotactic factor of Anaphylaxis (ECF-A) :

History : ECF-A was originally discovered in crude anaphylactic effusate by Kay et al. (1971), in guinea pig lungs and by Kay and Austen (1971) in human lungs. Subsequent extraction studies revealed that ECF-A is present preformed in rat mast cells, mast cell rich respiratory human and guinea pig tissues (Wasserman et al., 1974), nasal polyps (Kalinor et al., 1973), polymorphonuclear leukocytes (Czarnetzki et al., 1976), etc.

Chemistry and Biosynthesis :

ECF-A is a small molecular weight (500-1000) (Kay and Austen, 1971), acidic peptide (Czarnetzki et al., 1976). Its chemical structure and mode of biosynthesis is not known.

Storage and Release :

ECF-A is granule associated in the rat mast cells along with the histamine (Wasserman et al., 1974) in preformed state (Czarnetzki et al., 1975). It is also present in human basophils, neutrophils and eosinophils (Czarnetzki et al., 1976). ECF-A release from human lung and nasal polyps by IgE-dependent direct or reversed reactions has the same biochemical requirements as histamine release, and is similarly modulated by prototype adrenergic and cholinergic receptors via alterations in intracellular concentrations of cyclic nucleotides (Kalinin et al., 1973; Wasserman et al., 1974).

Pharmacological effects in Anaphylaxis :

During localised anaphylaxis, aggregation of a variety of leukocytes is observed. This is due to chemotaxis induced by ECF-A (Auston, 1973). The role of ECF-A is not yet very clear, however, it might be responsible for persistence of the effects of anaphylaxis and be co-operative to other factors. They cause increased antibody binding to cells (Capron et al., 1981).

Chapter - 2

TREATMENT OF ASTHMA:

1. Review of Present Treatment for Asthma :

Due to the diversity of the causes of asthma, many types of antiasthmatic drugs are used in the clinic. Some newly understood mechanisms in asthma have further necessitated the need of the development of new drugs to make the treatment more effective and specific. The preliminary aim of the long term treatment is to maintain the patient as near to his normal pulmonary status for as much time as possible. This can be achieved by using multiple therapeutic approaches, that can be categorised in three major groups as :-

- i) General measures in asthmatic treatment.
- ii) Pharmacological treatment.
- iii) Immunological treatment.

1) General measures (Weiss, 1975) :

Health, Environmental Control and Avoidance therapy : A nourishing, nonallergic diet, liberal fluid intake, adequate rest and sleep, reasonable physical activity and exercise, are essential to keep the patient

healthy. Exposure to factors playing a role in the etiology of asthma as pollens, dust, infection, dampness, fatigue, inhalation of offensive chemicals, should be avoided.

Psychologic management : Stress and serious emotional disturbances in asthmatic patients should be avoided as they may increase the severity of attacks or some times themselves precipitate an attack. A pleasant environment with an understanding of the patient's psychology should be created.

ii) Pharmacological Treatment :

Theoretically, pharmacological intervention is possible, to overcome an asthmatic attack directed towards several systems (Wilson and McPhillips, 1978) as :-

- a) Antibody formation,
- b) Antigen-antibody reaction,
- c) Alpha-adrenergic and cholinergic system,
- d) Beta-adrenergic system,
- e) Mediator release,
- f) Mediator action,
- g) Phosphodiesterase enzyme (Cyclic-AMP level).

In practice, mainly four groups of agents are used to control asthma (Pain, 1973) :-

- 1) Sympathomimetic amines (Bronchodilators).
- 2) Xanthine derivatives (Phosphodiesterase inhibitors).
- 3) Corticosteroids (Antigen-antibody reaction interfering compounds).
- 4) Sodium cromoglycate and similar compounds (Mast cell stabilisers).

In addition to these other miscellaneous agents as antihistaminics, alpha-adrenergic and cholinergic antagonists are also useful to some extent in individual cases.

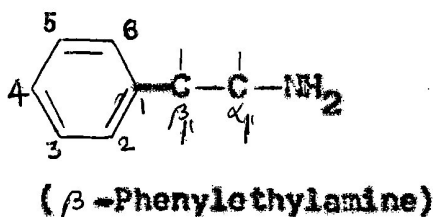
Sympathomimetic amines (Bronchodilators) :

Sympathomimetic amines in their crude plant extract forms were used for centuries in pharmacological control of asthma. Barger and Dale (1910) first studied the pharmacological activity and structure activity relationship of a large series of synthetic amines related to epinephrine and termed their action as sympathomimetic. Although these amines differ from one other in several characteristics as chemical structure, absorption, metabolism, etc. the basic mechanism of action of these drugs is the same. They affect many body processes, the bronchodilator effect of which, is

unequivocally useful in asthma, while other effects as cardiac stimulation, other smooth muscle relaxation (gut, blood vessels, etc.) are undesirable.

Chemistry :

β -Phenylethylamine is the parent structure of all sympathomimetic amines.



Substitutions on α and β carbon atoms, terminal amino group and the aromatic ring are possible which yield different compounds with sympathomimetic activity (adrenergic receptor activity). The degree of alpha and/or beta adrenergic receptor activity of a compound is mainly dependent on this substitution. As the effect of sympathomimetic amines on bronchial smooth muscle is mediated through beta-2-adrenergic system, a specific beta-2 selective compound is preferable in the treatment. To develop a beta selective compound -

i) the size of alkyl substitution on amino group can be increased (e.g., isoprenaline),

ii) the presence of 3,4-hydroxy groups on phenyl ring is essential for maximal activity, but 3,5-dihydroxy

phenyl rings with large amino group substitution confers beta-2 receptor selectivity (terbutaline),

iii) the oral effectiveness of a compound and its duration of action can be enhanced by deleting one or both hydroxy groups on phenyl ring with presence of α -carbon-methyl group,

iv) groups other than hydroxy on aromatic ring decreases the activity, and

v) β -Carbon substitution increases both alpha and beta receptor activity.

By using these and other guide-lines compounds having selectively beta-2 receptor action can be developed.

Pharmacology :

Epinephrine, ephedrine and isoprenaline have been used for a long time to relieve the bronchospasm due to an asthmatic attack. Lande et al. (1967), divided the beta adrenergic receptor system in two sub-groups as beta-1 and beta-2, and identified that the tracheal smooth muscle relaxation is mediated through beta-2 adrenoceptor system. A new generation of compounds soon came forth, with almost specific beta-2 adrenoceptor agonist activity.

Epinephrine, though possesses a rapid onset of action, its both alpha and beta adrenoceptor actions limit its usefulness as a bronchodilator, with the emergence of new specific beta-2 agents. Epinephrine with its alpha receptor action, constricts the bronchial mucosa vessels, and may reduce the congestion and oedema. But this may form a more viscid mucous plugs, difficult to dislodge and thus, increasing the respiratory distress. Some patients also show refractoriness to epinephrine treatment.

Ephedrine, when used alone, is a weak bronchodilator and is used in combination with other drugs as theophylline, to be more effective. Though Weinberger and Bronsky (1975) demonstrated that the combination did not have any apparent advantage over theophylline therapy alone and the side effects of ephedrine are enhanced in the combination. The main drawbacks of ephedrine therapy are CNS stimulation, tolerance, nervousness, tremors and tachycardia.

Isoprenaline (isoproterenol, $dl-\beta$ -[3,4-dihydroxyphenyl]-isopropyl amino ethanol) is the first sympathomimetic amine found to be having exclusive beta-receptor action and almost no action on alpha receptors. Konzett (1940) first studied its effects, which was followed by numerous studies to establish it as a

bronchodilator. Almost all varieties of smooth muscles are relaxed by isoprenaline, with most pronounced activity on bronchial and gastrointestinal muscles. The bronchospasm in asthmatics is prevented or relieved by isoprenaline, by its beta-receptor stimulant action, which may be supported by its inhibitory action on antigen-antibody reaction (Assen and Schild, 1969). It is effective by inhalation, injection and sublingual routes. Orally it is ineffective as it is metabolised by catechol-O-methyl transferase either in intestinal wall or liver following absorption. The duration of its response is short and the margin of safety is very low with a high incidence of side effects as tachycardia, tremors, palpitations, hypoxemia, etc. The excessive use of isoprenaline from metered dose inhaler have caused deaths in certain patients (Spoizer *et al.*, 1968a; 1968b). All these side effects can be minimised under controlled recommended clinical dose.

A new series of compounds, collectively called as beta-2 selective agents, resulted from synthetic modification of isoprenaline moiety with almost specific beta-2 adrenergic action, less beta-1 adrenergic action and practically no alpha-adrenergic action. Some significant compounds of this series are salbutamol (Cullum *et al.*, 1969), carbutolel (SK & F 40383A) (Wardell *et al.*,

1974), bitulterol (Minatoyo, 1978), terbutaline, feneterol (Stepanock, 1978), practerol (Yamashita et al., 1978), metaproterenol, etc. Almost all of these compounds are active orally and with a long duration of action. Though side effects of isoprenaline could not be completely eliminated from these drugs at least their bronchodilator activity is far more enhanced with relatively little cardiac stimulation (Legge et al., 1971). Several workers like O'Donnell and Wanstall (1974), Malta and Raper (1974), Wagner et al. (1973), have showed in vitro that these beta-2 selective drugs have a relative preference of beta-2 receptor over cardiac receptor with salbutamol being most selective (selectivity index 250) and isoproterenol least (s.i. 1.4). In vivo, similar results were demonstrated in dogs (Wasserman and Levy, 1974), cats (Wagner et al., 1973), man (Svedmyr and Thiringer, 1971). The tremors are more severely caused by these drugs than isoprenaline and at present it is not possible to separate the bronchodilator and tremor enhancing property of beta-2 adrenoceptor agents (Yamashita et al., 1978).

Mechanism of action :

Reactions of sympathomimetic agents on tissues which respond to them is initiated by an interaction of the agent with the beta-adrenergic receptors. Robinson

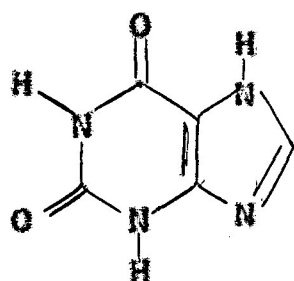
et al. (1967) suggested the beta-adrenoceptor to be a molecule of adenyl cyclase. The reaction of agent with beta-adrenergic receptor leads to the activation of the enzyme which hydrolyses ATP to cyclic AMP. Increased intracellular concentrations of cAMP leads to activation of protein kinase system, which produces a response via a chain of biochemical events, not fully understood.

Xanthine Derivatives (Theophylline group of compounds) :
(Phosphodiesterase inhibitors) :

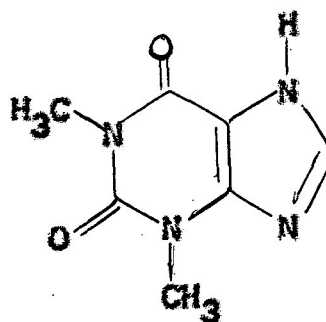
The alkaloid theophylline occurs in plants, of various *Thea* species, and mainly *Thea sinensis*. These plants containing xanthine derivatives are known to man since long and are used to make beverages. Theophylline derivative, aminophylline, was first reported on the successful treatment of status asthmaticus by Harmann and Aynesworth (1937) and since then theophylline compounds are used in treatment of asthma.

Chemistry :

Theophylline is a methylated xanthine derivative with a dioxypurine parent nucleus. It is 1,3-dimethyl xanthine.



(Xanthine)



(Theophylline)

The theophylline derivatives with small non-polar groups substituted at 1 or 3 positions are more potent in inhibiting the cyclic nucleotide phosphodiesterase enzyme. A benzyl group at 7 position and substitution of 6-O with a S further enhances this activity. An addition of aliphatic group at position 8 also gives more activity. In spite of these studies no synthetic derivatives of xanthine are yet emerged to challenge the therapeutic use of theophylline or aminophylline.

Pharmacology :

The xanthines relax various smooth muscles. Particularly it relieves the bronchospasm produced either experimentally, by autocoide as histamine, acetylcholine or clinically in asthma. Theophylline is most effective in this respect. Bertelli *et al.* (1973) claimed that theophylline act synergistically with beta-2 adrenergic agonist, relaxing airway muscle preparation in vitro. But Wolfe and Colleagues (1978) failed to show such synergistic action. Intravenous aminophylline or theo-

phylline are still used as major drugs in management of asthma, as a prophylactic agent or along with sympathomimetic amines. The absorption and metabolism rate of theophylline shows a wide variation in different individuals, which changes its plasma levels accordingly. The high plasma levels may lead to life threatening convulsions, with pulmonary and cardiovascular collapse (Persson and Erjeflat, 1981). But the controlled levels of theophylline between 10-20 $\mu\text{g/ml}$ provide substantial improvements in pulmonary function with minimum side effects.

The untoward effects of theophylline are irritability, dehydration, severe vomiting, hematemesis stupor and convulsions (Solfer, 1957; Persson and Erjeflat, 1981), mostly due to its central stimulating action. The nausea and vomiting is produced more by oral route.

Mechanism of Action :

The enzyme phosphodiesterase which is a modulator of intracellular cyclic AMP concentrations is inhibited by xanthine derivatives. This inhibition causes an accumulation of cAMP which leads to relaxation of bronchial smooth muscle and thereby the relief in the obstruction (Schulz et al., 1973). The other

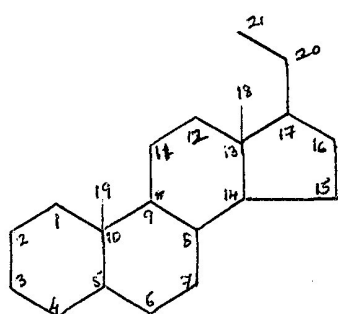
actions of methyl xanthines as potentiation of inhibitors of prostaglandin synthesis (Vinegar et al., 1976), and reduction of the uptake and/or metabolism of catecholamines by them in non-neuronal tissues (Kalsner et al., 1973), are some neglected areas, worth studying for their involvement in the mechanism of action in asthma.

Corticosteroids (Antigen-antibody reaction inhibitors):

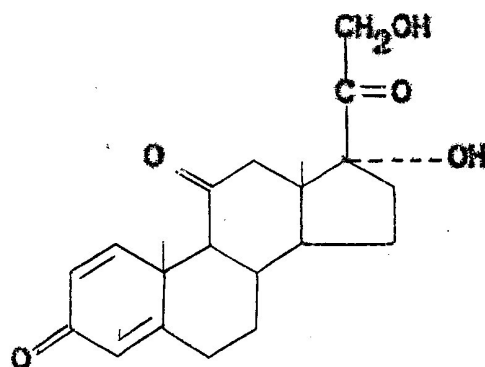
Bordley and co-workers (1949) first described corticosteroids as beneficial in asthma. They can be most favourably used as long term treatment in chronic asthma when other drugs fail, as they restore an almost normal respiratory function and minimise the chance of death. In present therapy they are probably the most reliable, effective agents, and if they were free of side effects, they would have been used as drug of choice in asthma.

Chemistry :

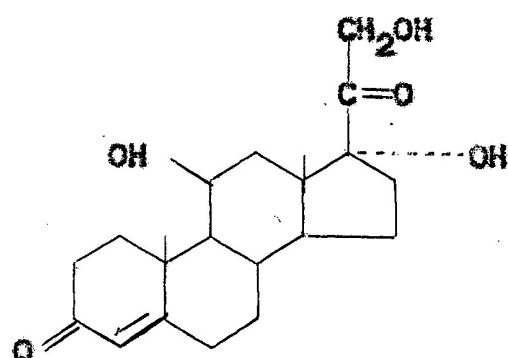
Corticosteroids are 21 carbon steroids and described as derivatives of pregnane or androstane. The basic structure of all steroids is perhydrocyclopentanophenanthrene ring system. Steroids cortisol, prednisone, beclamethazone are used mainly as anti-asthmatic agents.



(Perhydrocyclopentano-phenanthrene)



(Prednisone)



(Cortisol)

Pharmacology :

Corticosteroids by their antiinflammatory activity reduce the oedema and they also potentiate the activity of beta-agonists (Shenfield et al., 1975). They produce effects within 1-12 hours of administration. They are administered systemically (oral or intravenous) or as inhalations in severe or acute asthmatic attacks not responding to other agents. As they show a number of side effects they are added as the last therapy programme, if possible with the removal of old regimen. Intravenous hydrocortisone for acute attacks and oral

prednisone for severe chronic attacks in smallest dose, are the usual choice, as their half life is shorter than that of other corticosteroids and hence they cause minimum adrenal suppression. Clarprednol, a new agent, is also used with same purpose. Introduction of beclomethasone inhalation was one of the advances in chronic asthma treatment as it showed excellent topical activity with least side effects (Brogden et al., 1975). Beta methasone and dexamethasone are also used as inhalations but are less effective than beclomethasone.

The major side effects of chronic corticosteroid therapy are their hindrance with the normal endogenous adrenal cortical functions as well as corticoid over-stimulation leading to osteoporosis, glucose intolerance, delayed healing, etc. Children show some times growth retardation. The sudden withdrawal of the drug, may lead to rheumatic and other complaints and hypotension (Kaufman et al., 1976), hence withdrawal should be done gradually.

Mechanism of Action :

The exact mechanism of action of steroids in relieving an asthmatic attack is not yet clearly understood. They act by their anti-inflammatory property to reduce the oedema, but they also stimulate responsive-

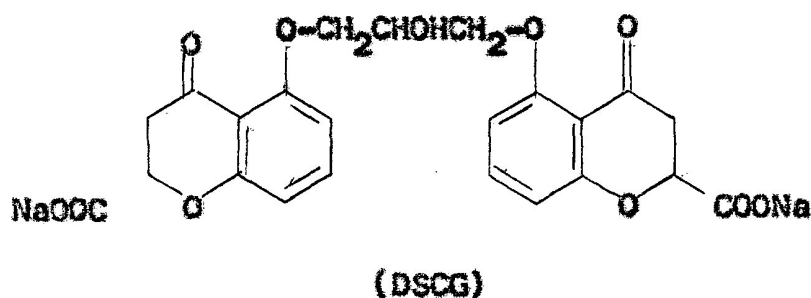
noss to bronchodilator therapy which had become unresponsive before their treatment. Steroids are said to act by inhibiting the antigen-antibody reaction, affecting almost every phase of the immune response. However, there is no convincing evidence regarding the mechanism of this immunosuppression. The therapeutic doses of steroids have no significant effect on the circulating IgG or IgE antibody titres, also metabolism of complement is not significantly affected (Claman, 1975). Thus, in spite of the fact that the symptoms of disease are often cured dramatically by steroids, their exact mechanism is yet to be deduced.

Sodium cromoglycate (Mast cell stabilisers) :

Cox and his colleagues working from 1956 , to develop a potent and long acting antiallergic agent from khellin (with mild bronchodilator activity), found that one of the compounds of the bischromone series, FPL 670 (disodium cromoglycate, DSCG, Intal) was effective in reducing the asthmatic response to inhaled antigen in sensitised subjects, with persisting effect. Later on, it was shown that the drug has no bronchodilator activity of its own but can prevent allergic broncho-spasm by inhibiting the autocolid release as a result of antigen-antibody reaction (Cox et al., 1970).

Chemistry :

Disodium cromoglycate (DSCG) is a bischromone, the disodium salt of 1,3-bis(2-carboxycromon-5-yloxy)-2-hydroxypropane, synthesised from 2,6-dihydroxyacetophenone.



Many other compounds with related cromone moiety like FPL-52791 (cromone-2-carboxylic acid series) (Sanno, 1977), PRD-92-EA (Devlin, 1977), doxantrozole (Tateson, 1976), Compound A (Johnson and Van Hock, 1973), BM 15,100 (Roesch and Roesch, 1976), H^1_5 (Koda et al., 1976), etc. were also found to be active as antiallergic drugs either experimentally or clinically, with the mechanism of action as DSCG. Some of the compounds like FPL-52791 are 12-folds as active as DSCG (Augstein et al., 1976).

Pharmacology :

DSCG, per se, does not relax any smooth muscle, nor does it inhibit significantly of the bronchoconstriction caused due to different spasogens (Cox et al., 1970).

But it inhibits the histamine, SRS-A and other autocooids release from the mast cells of lung tissue during allergic response mediated through IgE antibody (Spicer et al., 1975), and thereby reduces the severity of bronchospasm. This effect of inhibition on the antigen-antibody induced release of autocooids, can also be seen in inhibition of release of autocooids induced by compound 48/80, dextran, calcium ionophore A-23187, etc. (Johnson and Back, 1975; Marshall, 1972).

The acute toxicity of DSCG is low. The drug is well tolerated and adverse reactions are minor. In inhalation preparation direct irritant effect of powder may produce bronchospasm, wheezing, cough, nasal congestion, pharyngeal irritation, etc. some times dizziness, headache, joint pains, nausea, allergic rash may be seen in individual cases. More serious and rare side effects are hypersensitivity to drug, laryngeal oedema, anaphylaxis, etc. (Douglas, 1980). Though Beach and co-workers (1981) in their studies on DSCG toxicity in primates demonstrated an adequate safety margin between therapeutic dose of DSCG and the lowest dose to produce the toxic effects. The main objection to DSCG treatment is that in about 50% of the patients it shows no significant improvement and to check the effectivity of the treatment long periods are required (Pain, 1973).

According to Brogden et al. (1974) refractiveness of half the patients might be due to the cause that DSCG is more effective in extrinsic asthma, where allergy is root cause, than in intrinsic asthma, or infectious asthma. The other drawback is that DSCG is not itself a bronchodilator hence cannot be used in acute attacks or during an attack but must be used as a prophylactic agent.

Mechanism of Action :

DSCG is believed to act on the pulmonary mast cells, to stabilise their membrane so that autocoids like histamine, SRS-A, etc. will not be released during an anaphylactic reaction. Inhibition of antigen induced release of histamine, formation and release of SRS-A and other autocoids from rat peritoneal mast cells by DSCG is demonstrated by many workers (Brogden et al. , 1974; Orr et al., 1971). It does not inhibit the binding of IgE antibody to mast cells or the interaction between cell bound IgE and specific antigen, but the secretory response to this reaction is rather suppressed by it (Goose and Blair, 1969). The exact mode of this suppression is not yet clear but according to Theoharides and colleagues (1980) it does so by regulation of phosphorylation of a mast cell protein. This is vaguely termed as membrane stabilisation action of DSCG on the

mast cells. Dixon et al. (1979) suggested other mode of action as it produces reflex hypotension and thus reverse histamine induced reflex bronchospasm by activating receptors in the left ventricle of heart.

Miscellaneous Agents :

a) Alpha-Adrenoceptor Blocking Drugs :

The bronchoconstriction caused by alpha-adrenergic stimulation supports the theory of use of alpha-adrenergic blockers in control of asthma to some extent. These agents have been shown to be moderately effective in producing bronchodilation in asthmatics (Patel and Kerr, 1975). Alston and his colleagues (1974) showed that alpha-receptors blocking drugs like thymoxamine and phentolamine can be used effectively in some patients showing resistance to beta-sympathomimetic treatment. The drugs have got a short duration of action and cause irritation when inhaled. These drugs have very limited use in asthma therapy when used alone, but their combination with beta-receptor stimulants like isoprenaline, salbutamol, may find a place in management of asthma (Patel and Kerr, 1975).

b) Anticholinergic agents :

The predominant role of parasympathetic nervous system in regulation of airway calibre was shown in dogs

by Cebezas et al. (1971). The vagally mediated reflex bronchoconstriction due to stimulation of irritant receptors, by many non-antigenic physical or bronchoactive agents in man and in animals was also shown by Nadel et al. (1965), DeKock et al. (1966). Atropine and its derivatives like ipratropium bromide, methascopalamine are reported to prevent this bronchoconstriction (Ruffin et al., 1977), if not completely at least a part of it (Cropp, 1975).

Atropine in inhaler form is more acceptable than systemic route because its side effects such as cycloplegia, mydriasis, urinary retention, tachycardia are seen to less extent.

Ipratropium bromide (Atrovent) has a bronchodilator activity comparable with isoprenaline and possesses a longer duration of action and negligible side effects (Yeager et al., 1976).

The efficacy of methascopalamine (Pamine) against methacholine induced bronchospasm in asthmatics was prolonged, without cardiovascular side effects, but the drying of mucosa is seen.

c) Mediator Inhibitors :

1) Antihistaminic agents : Antihistaminics like chlorpheniramine, are shown to be beneficial intravenously, at higher doses, particularly against the immediate phase of dual type anaphylactic reaction in asthma (Nakazawa et al., 1976). The mechanism of action by which the antihistamine interferes with release of process is not yet known. These agents need further investigation in terms of their ability to inhibit histamine release rather than their ability to block the effects of released histamine (Lichtenstein, 1973).

2) SRS-A Inhibitors : The SRS-A inhibitor, Diethylcarbamazine, has found to be ineffective in asthma largely but in few cases of exercise induced asthma in children it may be of some benefit (Sly and Matzen, 1974).

3) Prostaglandins : Though prostaglandins of F series (PGF_2) are the mediators release by allergic, chemical or physical stimuli causing bronchoconstriction, prostaglandin E (PGE_2) is reported to be bronchodilator and PGF inhibitor (Piper and Vane, 1969). By inhalation route it is irritating and not more effective than other non-irritating agents (Mathe and Hedqvist, 1975). Intravenously it is rapidly destroyed before

reaching the appropriate site. Hence it finds no place in asthma treatment.

iii) Immunological Treatment :

Hyposensitisation : Noon (1911) first suggested the specific desensitisation in allergic states. Loveless (1940) showed that repeated administration of minute amounts of allergen stimulates the production of heat stable IgG antibody (blocking antibody). This antibody has the capacity to reduce skin responses to allergens and patients having high titres of blocking antibody appear to have better clinical response to hyposensitisation. As blocking antibody rises, levels of IgE tend to fall, and the rise of the latter during the pollen season tends to be less. At the same time, during desensitisation, cell sensitivity tends to fall (Ishizaka and Ishizaka, 1973).

The clinical trials of desensitisation in asthma are difficult to interpret or to summarise, because there are so many variables of clinical selection methods, for monitoring results and dosage regimens. A small number of carefully performed studies do suggest that desensitisation to pollen may be helpful in Asthmatics (McAllen, 1961). But the results reported are inconsistent.

2. Review of Plant Products as Antiasthmatic Agents :

In Ayurveda a number of plants have been claimed to be useful in "Svasakasam" (Respiratory diseases). Nadkarni (1976) in his book "Indian Materia Medica" has described about 115 different remedies from Ayurvedic, Siddha and Unani medicines either of plant or non-vegetable origin, as antiasthmatic agents, alone or in combination with other extracts or ingredients.

In the "Glossary of Indian Medicinal Plants" Chopra et al. (1956) also have listed more than 150 plants of Indian origin, referred in Ayurveda and other old Indian medicinal literature, for their use in asthma and other pulmonary disorders. Though not all, quite a few of these plants are evaluated either experimentally and/or clinically, for their pharmacological activity, and proved to be useful in asthmatic and/or allergic conditions. In most of the cases the work was in primary stages. A pharmacologically active compound was isolated from a few plants as *Adathoda Vasaka*, *Swertia chirata*, etc., while all other studies are done on extracts of whole plant or a part of it.

In the following parts of this section, only a few such plants which are proved experimentally and/or clinically to be active or potential as antiasthmatic

agents are reviewed briefly. At the end of the section some plants which are proved to be antiasthmatic and/or antiallergic in primary studies and are worth pursuing are listed in Table - 2.

1) Adhatoda vasaka (Acanthaceae) (Vasaka, Adulsa) :

This plant has been studied extensively by many workers for its chemical and pharmacological evaluation. The leaf extract of the plant was used in India against several respiratory disorders since long time (Chopra, 1956), and it comes in several proprietary medicines under the popular name vasaka syrup. The plant contains alkaloids of which Amin and Mehta (1959) reported a large amount of vascicine mixed with small amount of vascicinone, its oxidised product. They attributed the beneficial action of extract in respiratory disorders to vascicinone either present already or formed by auto-oxidation of vascicine. Later, these two alkaloids were studied in detail for their pharmacological effects, and it was found that vascicinone is a bronchodilator, weak cardiac stimulant and a potent antianaphylactic agent, while vascicine is a weak bronchoconstrictor, cardiac stimulant and devoid of antianaphylactic activity (Bhide et al., 1974; 1976).

A suggestion for use of preparation of vascicinone

alone than that of traditional vasaka extract (which contains unknown proportions of two oppositely acting alkaloids) was made to avoid the interfering effects of vascicine (Naik et al., 1975). The acute chronic treatment studies of vascicinone was done and the antianaphylactic activity was shown to be comparable with DSCG (Bhide and Mahajani, 1975), with probable mechanism of action by increase in cellular cyclic AMP levels, through inhibitions of phosphodiesterase enzyme activity (Bhide et al., 1974).

ii) Ammi Visnaga (Umbelliferae) (Kholla, Khilla) :

Khellin, the most important active constituent of the plant is a coumarone derivative in crystalline form (Huttrer and Dale, 1951). Anrep et al. (1947; 1949) reported its bronchorelaxing effect, but its main action is antispasmodic, diuretic, and coronary vasodilation. It also has been reported to be of use in bronchial asthma (Anrep, 1947).

Though Khellin has got no place in the asthma therapy, DSCG, one of the important compounds in asthma treatment, has resulted from a long term work of Cox and his colleagues, while working on Khellin and its derivatives (Cox et al., 1970).

iii) Cassia siamea (Leguminosae) (Kassod) :

Bhide (1980) reported antianaphylactic activity of a compound (substance H), isolated from the plant, in different laboratory models as passive cutaneous anaphylaxis, passive peritoneal anaphylaxis, etc. The histamine inhibition ranged from 70-90% of the activity of disodium cromoglycate.

As the yield of the substance H from the plant was very poor their, their collaborating chemists synthesised a series of compounds analogous to substance H. Of these compounds, compound E was found to be most active and its detailed laboratory studies were published by Bhide et al. (1976). Compound E was active orally and was a potent antianaphylactic agent, more active than disodium cromoglycate, but with no bronchorelaxant activity.

iv) Ephedra (Ephedraceae) (Ma Huang) :

This plant was known in China over two thousand years ago, under the name of Ma Huang. The chief constituent ephedrine, an alkaloid from various species of ephedra (like E. sinica, E. equisetina, E. intermedia, etc.) is useful in asthma. The alkaloid has been reviewed in detail in previous section.

v) Mesua ferrea Linn (Guttiferace) (Nagkesar) :

The plant has been used in Unani system of medicine and a wide range of diseases are claimed to be cured by the medication with this plant. Its leaves, flowers and seed oil are described for various uses. It was also used for cough attended with expectoration. Bhide et al. (1977) demonstrated the antianaphylactic activity by the phenolic fraction of the seed oil, however, the fraction was not active as a broncho-dilator agent.

vi) Picrorhiza kurroa Royle (Schrophulariaceae)
(Katuka, Katki) :

A glycoside from the plant picrorhizin is reported. Picrorhiza is mainly used as bitter tonic and laxative. Mahajani and Kulkarni (1976), reported an antiasthmatic activity of the root powder of the plant. They demonstrated the chronic treatment of the powder, reduced the total histamine contents of the lung and also the inhibition of release of histamine and SRS-A.

vii) Piper longum (Piperaceae) (Pippali) :

Fruits and roots of the plant have been claimed in Ayurveda to cure respiratory disorders. Athavale and Fernandes (1980) reported the usefulness of plant in asthmatic children. Dahanukar and Karandikar (1981)-

extended their work and studied the fruit powder of the plant on standard antiasthmatic models as well as clinically. Their reports suggested that the plant possesses antianaphylactic activity with no significant bronchorelaxant activity. The drug was also effective clinically.

viii) Swertia chirata and decussata (Gentianaceae)
(Chireta) :

The plant was studied by Ghosal et al. (1973) for its chemistry. They isolated nine xanthenes and several other compounds from the plant. Ghosh and Bhide (1930) showed the antianaphylactic activity of the total acidic and phenolic fraction of the plant on a new model. Later (1981) the group isolated the xanthenes as described by Ghosal et al. and identified that xanthenes-IV and VII possess the antianaphylactic activity, while other xanthenes are inactive. The compounds are reported to cause no bronchorelaxation in animals except at very higher doses but as antianaphylactic agent it is more potent than disodium cromoglycate.

ix) Thea sinensis (Theaceae) (Tea, Chai) :

The main constituents of the plant, methyl xanthines and especially theophylline is reviewed in the previous section.

x) Tylophora indica/asthmatica (Asclepiadaceae)
(Ananto mul) :

The Tylophora plants have been used in India since long in treatment of bronchial asthma. The presence of a number of alkaloids and mainly a mixture of two alkaloids - Tylopherine and Tylophorinine in the leaves of the plant was reported by Govindachari and co-workers (1954). A review of these alkaloids is published by Govindachari (1973).

The value of plant in asthma was studied by Shivpuri et al. (1969). The plant enjoyed vast popularity for a few years in clinical allergy as a result of the work of Shivpuri (1973; 1975) and Gupta (1975). Bhide et al. (1974) showed a bronchodilator as well as anti-anaphylactic activity to the chloroform extract of the leaves of I. indica. Though efforts to isolate the pure active fraction have failed so far.

Table - 2 : Some plants having antiasthmatic/antianaphylactic activity.

No.	Plant	Active compounds/ extracts	Activity	Reference
1.	<u>Cleorodendron serratum</u> (Bharangi)	Glucoalkaloidal saponins.	Mediator inhibitors	Gupta & Gupta (1967)
2.	<u>Curcuma longa</u>	Alcoholic extract	Antianaphylactic	Gupta, (1971)
3.	<u>Gardenia latifolia</u>	Saponins	Antianaphylactic	Gupta, (1974)
4.	<u>Gardenia turgida</u> (Thanolla)	Saponins	Antianaphylactic	Gupta, (1971)
5.	<u>N. jatamansi</u>	Alkaloidal extract	Mediator inhibitors	} Gupta <u>et al.</u> (1962; 1963); Gupta (1971)
6.	<u>Rhus succednea</u> (Kakrashringi)	Alkaloidal extract	Mediator inhibitors	
7.	<u>Saussera lappa</u> (Kuth)	Saussarine (alkaloid)	Bronchiolo relaxant	Chopra & De (1929).
8.	<u>Solanum xanthocarpum</u> (Kantkari)	Glucoalkaloidal saponins	Mediator inhibitors	Gupta & Gupta (1967)

Chapter - 3

REVIEW OF PLANT BUTEA MONOSPERMA (LAZI) - KUNTZE :

Syn. - B. frondosa Koenig ex Roxb.

Family - Leguminosae.

Common names - The flame of the forest, Palas, Palash, Parasa, Khakra, Moduga, etc. (Nadkarni, 1976; Wealth of India, 1948).

The plant is a medium sized deciduous tree, 10-15 feet in height and 5-6 feet in girth. The bark is greenish-brown to light brown and yields a ruby-red gum. Flowers are orange-red, blooming at the beginning of summer season. The pods contain a single seed at its apex.

Palas is common throughout India, Burma and Ceylon. It is frost-hardy and drought resistant and a valuable species for reclaiming saline soil. Plantations can be raised both on irrigated and dry lands. In India it is mainly used as a host tree for lac insects. Although quality of the lac produced is inferior, the quantity is greater than in any other species.

Gum : The vitreous, ruby-red gum exuded from natural cracks or artificial bark incisions, is known as "Butea gum" or "Bengal kino". It is a powerful astringent

and given in many forms of chronic diarrhoeas. Fresh juice exuded is also used as application in ulcers and congested or septic sore throat.

Bark is given with ginger in snake bites. A weak decoction of bark is useful in catarrh, cough and cold.

Roots are of no value except for preparation of fibres.

Wood is of no much use except as fuel, cheap board wood and to a small extent in structural work.

Leaves are mainly used to prepare platters and cups, as cattle feed, and beedi wrappers. Medicinally, they are externally used to disperse boils, pimples, etc. and internally in flatulent colic, worms and piles.

Flowers are useful to make a yellow dye. They promote diuresis, menstrual flow and its poultice is used to disperse swellings.

Seeds powder is long been valued as anthelmintic when it is fresh, internally. Externally, paste of powder is used as a remedy for ringworm and herpes.

Chemistry :

Gum : Contains a large proportion of tannin and mucileginous material. On dry distillation it is reported to yield pyrocatechin.

Bark : Proanthocynidins (Tetramers of leuco-cyanaidins with C-C and C-O-C linkages) have been isolated from the gum and bark of Butea monosperma (Seshadri and Trikha, 1971).

Wood, Roots are not studied for their chemistry.

Leaves and Flowers : Bhaskara Rama Murti and Seshadri (1940; 1941; 1944) reported the presence of glucosides of flavanone and chalcones in the flowers and particularly presence of butein (a chalcone) and butin (a flavanone). Puri and Seshadri (1953) reported a new glycoside, isobutrin.

Seeds : Kalesaraj and Kurup (1967) reported presence of palasonin in the seeds of plant. Palasonin is a lactone $C_{16}H_{22}O_6$, with a decaline ring system, containing two carboxyl groups. Tuminkatti and Manjunath (1929) examined the fixed oil chemically present in the seeds.

Biological Work :

The anthelmintic activity of the palas bij (seeds) water extract have been reported by Rajapurkar et al. (1961). Kaleysaraj and Kurup (1967; 1968) reported palasonin as the anthelmintic active principle from the seeds. They also studied palasonin for its toxicity and other pharmacological effects on heart, smooth muscle, blood pressure, respiration and neuro-muscular conduction.

Sachdev et al. (1965) studied the toxicity of aqueous extracts of seeds of B. frondosa in rats, dogs and rabbits. Chronic administration by oral route for 2 months produced marked nephrotoxicity, and anaemia. Liver, lungs and spleen showed congestion. Stomach showed gross dilatation and chronic inflammation.

Clark and Geissman (1949a; 1949b) studied potentiation of epinephrine effects by flavanoid compounds (which included butin, butein and other 2',4,4'-dihydroxychalkone derivatives) on the isolated mammalian intestinal muscle and in intact animals. Rutin was used as a reference substance with unit activity. In this butein was less than 4 times but more active than Rutin but butin was less active than rutin.

PART III

MATERIAL AND METHODS

The work is divided in two parts :

Chapter-1 :

Phytochemical Work : Extraction and purification of the active fraction from the bark of Butea monosperma.

Chapter-2 :

Pharmacological Work : Pharmacological evaluation of the above mentioned fraction so as to establish its activity by using different in vitro and in vivo techniques, with special reference to the bronchodilator and/or antianaphylactic activity.

Chapter - 1

PHYTOCHEMISTRY :

MATERIAL :

The plant bark of Butea monosperma was collected from the E.S.I.S. Hospital campus, Dashi, New Bombay, in rainy season in July-Sept. 1979. Dr. R.P.Subhedar, Phytochemistry Department, Haffkine Institute, identified the plant.

Solvents :

1. Acetone,
2. Benzene,
3. n-Butyl alcohol,
4. Chloroform,
5. Ethyl acetate,
6. Methanol,
7. Methanol (AnalaR),
8. Petroleum ether.

All solvents used were of LR grade, supplied by
M/s Runa Industries, Dombivli.

Reagents :

1. Sodium hydroxide solution (1% and 10%).
2. Dilute hydrochloric acid (10%).
3. Sodium bicarbonate solution (5 %).
4. Ferric chloride solution in 0.5N hydrochloric acid (1-5 %).
5. Alcoholic solution of aluminium chloride (1%).
6. Dragendorff's reagent.
7. Mayer's reagent.
8. Glacial acetic acid) LR grade, Runa Industries,
9. Liquor ammonia) Dombivli.

Chemicals :

1. Sodium hydroxide pellets - Robert Johnson.
2. Sodium bicarbonate - Sarabha M. Chemicals.
3. Hydrochloric acid - Runa Industr. (Chemicals),
Dombivli.

4. Sulphuric acid - Runa Industr. (Chemicals), Dombivali.
5. Acetic anhydride - Glaxo Labs., Bombay.
6. Magnesium metal (foil).
7. Iodine (crystalline) - B.D.H., England.
8. Silica gel-G - Omega Chemicals, Thane.
9. Filter paper No.2 - Whatmans Ltd., England.

METHODS :

The bark of plant Butea monosperma was peeled out from stem, and dried in shade at room temperature, for one week. Dried bark was cut down to small pieces and ground to a coarse powder.

Dried coarse powder (0.5 kg) was extracted successively with 2.5 litres each of petroleum ether (40-60°C), chloroform and methanol, in a Soxhlet extractor. The solvent was changed over, when the solvent in the contact of powder became colourless. The extracts were concentrated in vacuum evaporator to a syrupy mass. Total 2 kg of the powder was extracted by this method in four lots, and comparable extracts from each lot were pooled.

Petroleum ether, chloroform and methanolic fractions were respectively designated as Fractions I, II and III, the total yield of each of them were 3 gm, 5 gm 10 gm respectively.

methanol (500 ml). The extract was partially soluble in cold methanol. Soluble (Fraction No.IV) (4.5 gms) and insoluble (Fraction No.V) (5.5 gms), parts were separated. The insoluble part was 5 times more active in both the models (as mentioned above) and was used further.

The insoluble fraction (5 gms) was dried and suspended in 1% sodium hydroxide solution (100 ml), resulting in a dark roddish brown solution (Fraction No.VI) with a small amount of greenish precipitate (Fraction No.VII). The solution was filtered and the precipitate (VII) (0.5 gm) was discarded as it was inactive. From the filtrate (VI) a dark brown mass was precipitated by adding 10 % hydrochloric acid, making pH acidic. The precipitate (Fraction No.VIII) was filtered, washed with distilled water to make it acid free and dried (1.5 gms). The filtrate, with wine-red colour (Fraction No.IX) was discarded.

Alternatively, so as to increase the yield of active fraction as well as to cut short the lengthy Soxhlet extraction procedure, a simpler method was followed to isolate the total phenols and acids from the plant. Powdered bark (350 gms) was extracted directly with 1% sodium hydroxide solution (500 ml) in cold, in a glass percolator. Percolate was a dark-red solution.

Percolation was continued by using fresh sodium hydroxide (300 ml) every time, till the colour of the percolate faded. All these extracts were pooled (Fraction A) (1.5 lits.) and 10 % hydrochloric acid was added to the pooled solution to make it acidic, when a dark-brown mass precipitated out. The precipitate (Fraction B) was filtered and made acid free by washing it with distilled water. The filtrate (Fraction C), was discarded.

The precipitate (Fraction B) was dried completely under vacuum to remove all water (3 gms). It was macerated with hot dry methanol (AnalaR grade), when a reddish brown solution in methanol was formed with a buff coloured residue. This solution was filtered hot and the buff coloured residue (Fraction D) was rejected. From the filtrate methanol was evaporated under vacuum, to give a dark red mass (Fraction E) (1.0 gm). Total 1.5 kg of bark powder was extracted by this method to give 4.5 gms of fraction E. The activity of fraction E was compared with the fraction VIII, isolated by the method, previously described. Both fractions showed equivalent activity on the two models used to assess the activity. These fractions also exhibited similar patterns in chromatographic studies. Hence, they were considered to be the same and pooled together (Fr. E-VIII) - Total yield 4.5 gms.

A crystalline sodium salt was formed from a part of fraction E-VIII (3 gms). Fraction E-VIII was completely dissolved in minimum amount of 0.1% sodium hydroxide solution to give a dark red solution with pH 7.2. The solution was evaporated to dryness in hot stream of air at 40-50°C. When sodium salt crystals of the total organic acids and phenols from the plant were obtained (Fraction F-X) (Wt. 2.5 gms).

The fraction F-X was used for detailed pharmacological studies by dissolving required amount of it, in distilled water or normal saline, just before the experiment.

Method II for separation :

In addition to isolate F-X, another method was followed for separation of active fraction. In this method methanol insoluble part (Fr. V), (1.2 gm) as in method I obtained from methanol extract of the bark from Soxhlet, was washed successively with petroleum ether (40-60°C) (1), ether (2), chloroform (3), ethyl acetate (4), acetone (5), methanol, (cold) (6), methanol, (hot) (7) and the residue remained was fraction 10. All these fractions were tested on both screening models to locate the fraction with maximum activity.

Chromatographic studies :

The fraction E, VIII and E-VIII were studied on thin layer and paper chromatographic techniques by using various solvent systems, so as to identify number of compounds present in active fraction.

Thin Layer Chromatography :

1) Preparation of plates : A thin layer of silica gel G, was formed on the glass plates (20 X 5 cm) by pouring the silica gel slurry, made in acetone, and spreading it by tilting the plates. The plates were activated in oven at 120°C for 1/2 hr.

2) Loading of fractions : The fractions were dissolved in hot methanol and applied on the plate with a capillary. Methanol was allowed to evaporate completely.

3) Development of plates : The plates were run in an air-tight gas jar in different solvent systems as follows :-

i) Petroleum ether, ii) Benzene, iii) Chloroform, iv) Ethyl acetate, v) Acetone, vi) methanol, vii) Chloroform-methanol (9:1), viii) Benzene-acetone (75:25, 50:50, 25:75), ix) Liquor ammonia (28% NH_4OH solution), x) Glacial acetic acid; xi) Methanol-ammonia (9:1), xii) Methanol:acetic acid (9:1), xiii) *n*-Butanol-

acetic acid:water (BAW) (4:1:5), xiv) 10% sodium hydroxide solution, xv) Saturated sodium bicarbonate solution.

Ascending paper chromatography of the fractions on Whatman No.2 filter paper, by using same solvent systems and chamber, were also carried out.

Visualization of the plates :

The resolved fractions were visualized with naked eye, and by putting the plates in iodine chamber.

Spot tests : The fraction E-VIII was studied for the presence of different chemical groups as alkaloids, flavonoids, etc.

1) Tests for presence of phenols and acids :

a) Phenols :

- i) Solubility in 1% sodium hydroxide solution.
- ii) Reaction with ferric chloride (1-5 %) solution in 0.5N hydrochloric acid (Fink and Fink, 1949).
- iii) Reaction with 1% alcoholic solution of aluminium chloride (Gage et al., 1931).

b) Acids :

- i) Solubility in 1% sodium bicarbonate solution.

2) Tests for presence of alkaloids :

a) Test with Dragendorff's reagent : (Munier and Macheboeuf, 1951) : On a filter paper a drop of concentrated hot methanolic solution was placed, solvent was completely evaporated. A drop of Dragendorff's reagent (solution A + solution B) was placed near the fraction spot such that boundaries of the two spots meet. Development of colour after mixing of the boundaries was noted.

b) Test with Mayer's reagent : (Cromwell, 1955): A drop of concentrated hot methanolic solution was placed on a glass slide and solvent was evaporated. On this spot a drop of conc. hydrochloric acid was put and mixed. Adjacent to this a drop of Mayer's reagent was put and two drops were allowed to mix. Formation of a precipitate at the interface of two drops or in the mixed drops was observed.

3) Test for presence of flavonoids : (Mabry et al., 1970):

Colour reactions with -

a) Aqueous sodium hydroxide solution : To 0.5 ml hot methanolic solution of the fraction, aqueous solution of sodium hydroxide was added and colour developed was noted.

b) Concentrated sulphuric acid : To 0.5 ml of hot methanolic solution of fraction, 1-2 drops of conc. sulphuric acid was added to observe the colour developed.

c) Treatment with magnesium/hydrochloric acid : To 0.5 ml hot methanolic solution of fraction a piece of magnesium foil and 1-2 drops of conc. hydrochloric acid was added and colour developed was noted.

4) Test for steroids :

Liberman and Burchard reagent test : (Burchard, 1989) : To 0.5 ml of hot methanolic solution of fraction one drop of acetic anhydride and 1-2 drops of conc. sulphuric acid was added and development of colour (particularly green) was observed.

5) Test for glycosides :

Mohli's Test : To fraction 10% -naphthol in alcohol was added and to this 2 ml of conc. sulphuric acid was added and development of red-violet ring at the interphase indicates the presence of glycoside.

Chapter - 2

PHARMACOLOGY :

MATERIAL :

Animals :

1. Mice - i) albino (NIH, Kasouli-Haffkine strain),
ii) 57/BL/6 strain,
2. Rats, albino (Haffkine strain),
3. Guinea pigs, (Haffkine strain),
4. Frogs,
5. Cats (stray).

Chemicals :

1. Sodium chloride (I.P.) - Sarabhai M. Chemicals.
2. Potassium chloride (Analar), B.D.H.
3. Dextrose (Analar), Glaxo Labs.
4. Sodium bicarbonate (G.R.) - Sarabhai Chemicals.
5. Sodium dihydrogen phosphate, $2H_2O$ (Analar) -
Sarabhai M. Chemicals,
6. Potassium dihydrogen phosphate (G.R.) -
Sarabhai M. Chemicals,
7. Magnesium chloride. $6H_2O$ (Analar), Glaxo Labs.
8. Magnesium sulphate. $7H_2O$ (G.R.) - Sarabhai M.
Chemicals.
9. Calcium chloride. $2H_2O$ (G.R. - B.D.H.

Physiological solutions (for constitution see Appendix- I) :

1. Normal saline (0.9 % w/v).
2. Tyrode solution.
3. Kreb's solution.
4. Feigon's solution.
5. De Jalon solution.
6. Frog Ringer solution.

Standard drugs :

1. Adrenaline - Gajjar's Pharmaceuticals.
2. Acetylcholine chloride - E. Merck.
3. Aluminium hydroxide gel - Gift from Hoechst Pharmaceuticals.
4. Aspirin - Alta Labs.
5. Atropine sulphate - C.H. Dörhinger Sohn.
6. Carbachol - Fluka Labs.
7. Carraegenan - Sigma Laboratories.
8. Chloralose - B.D.H.
9. Compound 48/80 - Sigma Laboratories.
10. Disodium chromoglycate (DSCG) - Fisons Pharmaceuticals Ltd., England.
11. Histamine Acid Phosphate - Sigma Labs.
12. Isoprenaline - Serva Feinbiochimica.
13. Leptazol - Bengal Immunity Co. Ltd.
14. Mepyramine Maloate -
15. Oxytocin - S.P. Pharmaceuticals.
16. Phenylbutazone - Ciba Geigy (India) Ltd.

17. propranolol - Sigma Laboratories.
18. Urethane - Merck.
19. Egg albumin - Gift from Hoechst Pharmaceuticals (Pvt.) Ltd.

Instruments :

1. Pocket thickness gage, Mitutoyo Inc., Japan.
2. Plethysmometer (Sequip).
3. Organ bath (INCO).
4. Kymograph (INCO).
5. Respiratory pump (C.F. Palmer (Lond.), Ltd.).
6. Displacement manometer (CF Palmer (Lond.) Ltd.)
7. Respiratory Tambour (INCO).
8. Square Wave Stimulator (INCO).

METHODS :

Different in vitro and in vivo pharmacological techniques were used to confirm the activity and mode of the action of fraction F-X. Guinea pig isolated tracheal chain preparation and compound 48/80 induced mouse hind paw oedema were used as preliminary test models to determine antiasthmatic activity, while other preparations were used as confirmatory models to show the bronchorelaxant and/or antianaphylactic activity of F-X. In addition to this several other general pharmacological experiments were carried out to determine other actions.

side effects and toxicity, if any, of F-X. Crude petroleum ether (I), chloroform (II) and methanol (III) fractions were also subjected to general pharmacological studies. In all in vitro and in vivo studies at least three experiments were performed except in analgesic, antiinflammatory and anticonvulscent testings, for which only one experiment was performed.

In vitro studies :

F-X was studied on -

- 1) Guinea pig tracheal chain preparation,
- 2) Guinea pig atria preparation,
- 3) Guinea pig ileum, and
- 4) Rat uterus.

F-X and Fractions I, II and III were studied on

- 5) Frog rectus abdominis preparation,
- 6) Rat phrenic nerve diaphragm preparation.

In vivo studies :

Fraction F-X was studied on -

- 1) Airway respiratory resistance in guinea pigs,
- 2) Compound 48/80 induced mouse hind paw oedema,
- 3) Active paw anaphylaxis,
- 4) Cat blood pressure and respiration, and
- 5) LD₅₀•

Fractions I, II and III were studied for -

- 1) Anti-inflammatory activity (Method - Carrageenan induced rat hind paw oedema),
- 2) Anticonvulsant activity (Method - Leptazol induced convulsions),
- 3) Analgesic activity (Method - Tail clip method).

In vitro Studies :

1) Isolated guinea pig tracheal chain (Castillo and DeBeer, 1947) : Guinea pigs (either sex, 250-300 gms) were stunned and bled to death. Trachea was isolated from the base of lungs upto pharynx and kept in Kreb's solution. The adherent tissues and muscles were separated and a tracheal chain preparation was prepared according to the technique of Castillo and DeBeer (1949). The preparation was mounted in a 10 ml organ-bath at 37°C with lower end fixed to the aeration tube and upper end connected to a frontal isotonic lever. The physiological solution used was Kreb's solution, with carbogen for aeration. The relaxation of the tracheal smooth muscle was recorded by the frontal lever on a kymograph.

Isoproterenol (isoprenaline) was used as a reference drug to cause relaxation of the tracheal smooth muscle. Dose-dependant relaxation of the smooth muscle

due to isoprenaline was first studied (dose range - 2-8 ng/ml). Effect of the fraction F-X per se or on the submaximal isoprenaline (2 ng/ml) relaxation of the smooth muscle was then recorded in dose range of 30-1600 μ g/ml of F-X. In case of isoprenaline and F-X doses, producing per se relaxation, the contact period with the tissue was 120 secs., while doses of F-X not producing per se relaxation the contact period was 5 mins., followed by a submaximal dose of isoprenaline (2 ng/ml) with 120 secs. contact period.

To prove the involvement of beta adrenergic system, a single dose of propranolol (5 ng/ml) was used as standard antagonist to block the responses produced by isoprenaline as well as F-X. The contact period of propranolol was 5 minutes followed by a submaximal dose of isoprenaline (2 ng/ml) and F-X (800 μ g/ml).

2) Isolated guinea pig atria (Clark, 1926) : The heart of the guinea pig (either sex, 250-300 gms) was removed immediately after stunning the animal and put in oxygenated Feigen's solution. The atria were isolated from ventricles and other tissues (aorta veins, etc.), and mounted in a 10 ml organ-bath containing Feigen's solution, with oxygen for aeration at room temperature. One of the tissue ends was fixed to aeration tube while other end to spring loaded sterling heart lever. The

beats of the atria were recorded on a kymograph by the lever.

Adrenaline was used as standard drug which caused an increase in force and rate of the contractility of atria, dose dependantly in the dose range of 5-80 ng/ml. The maximal effect of each dose was seen at 1.5 - 2 minutes after which adrenaline was washed out from the bath. Between two responses a rest period of 15 minutes was left.

F-X in the dose range of 200-800 μ g/ml was used and effects of it per se on the preparation and on sub-maximal doses of adrenaline (20 ng/ml) was studied. In the latter case F-X was added 5 minutes prior to adrenaline to observe its effects on adrenaline responses.

3) Isolated guinea pig ileum (Magnus, 1904) :

Guinea pigs of either sex (weight 250-300 gms) were killed by stunning, ileum was removed by opening the abdomen and was kept in Tyrode solution at 37°C. The lumen of the ileum was flushed with Tyrode's solution to remove semidigested food and mucous. One inch piece from the distal end of the tissue was mounted in a 10 ml organ bath, in Tyrode solution at 37°C with carbogen for aeration. The lower end of the tissue was fixed to the aeration tube, while upper end was connected to an

isotonic frontal lever which recorded contractions of the tissue on a kymograph.

Acetylcholine and histamine were used as reference drugs causing contractions of the ileal smooth muscle, dose dependently (both in the dose range of 50-800 ng/ml), with contact period of 30 secs. The recovery period between two responses was 2.5 mins.

Effects of fraction F-X per se or the submaximal contractions caused by acetylcholine (100 ng/ml) and histamine (100 ng/ml) were studied. F-X used in the dose range of 50-400 μ g/ml. The contact period of the fraction was 3 mins. before adding the standard agonists.

4) Isolated rat uterus (De Jalon et al., 1945) : Female rats (120-180 gms) were killed by stunning, uterus was isolated and placed in De Jalon's solution. Single horn of uterus, after opening the lumen of the uterine tube with a lateral cut, was mounted in a 10 ml organ bath, containing De Jalon's solution, with carbogen for aeration at 28°C. The lower end of the tissue was fixed to the aeration tube while upper end was connected to an isometric frontal lever which recorded contractions of the tissue on a kymograph.

Oxytocin and carbachol were used as standard agonist to cause a dose dependent contractions of the

tissue in the dose range of 1×10^{-3} i.u./ml and 1-16 $\mu\text{g/ml}$ respectively, with a contact period of 90 secs. Responses were repeated after every 5 mins.

Fraction F-X was studied on the preparation for its per se effects or for the interaction (potentiation or blockade), with oxytocin or carbachol submaximal doses. In the ⁺later case the contact period of F-X was 3 mins. prior to the submaximal response of standard agonist.

5) Isolated frog rectus abdominis preparation

(Burn, 1952) : Frogs (100-200 gms) were pithed and rectus abdominis muscle was isolated. By a nodial cut in the tissue two muscles were separated and a strip from one of the muscles was mounted in a 10 ml organ bath in frog Ringer solution with carbogen for aeration, at room temperature. The lower end of the tissue was fixed to the aeration tube while upper end was connected to a Gimbalever recording the contractions of the muscle on a kymograph.

Acetylcholine was used as standard muscle contracting agent, to cause dose dependent contractions of the muscle in the dose range 1-8 $\mu\text{g/ml}$ within 90 secs. The rest period between two responses was 5 mins.

Effects of fractions F-X and crude petroleum ether (I), chloroform (II) and methanol (III) extracts per se and on the submaximal contractions of acetylcholine were studied.

6) Isolated rat phrenic nerve diaphragm : (Bulbring, 1946) : Rats (either sex, 120-150 gms) were stunned and the phrenic nerve diaphragm preparation was mounted in a 40 ml bath, in Kreb's solution at 37°C with oxygen for aeration, by the method of Bulbring (1946). The indirect contractions of the diaphragm muscle were recorded on a kymograph with a starling heart lever after stimulating the phrenic nerve by two platinum electrodes. The nerve was stimulated at a voltage 2-4 V with 0.1 Hz frequency and 0.2 m.Sec. pulse width with a square wave stimulator.

Fraction F-X and crude petroleum ether (I), chloroform (II) and methanol (III) extracts were studied at a single dose of 400 µg/ml to cause any changes in the contractions of diaphragm muscle, induced by electrical stimulus, indirectly through nerve stimulation or directly, upto 15 mins. after addition of drugs.

In vivo Studies :

1) Airway respiratory resistance in guinea

pigs (Konzett and Rossler's Method, 1940) : Guinea pigs of either sex (300-350 gms) were anaesthetized with urethane (25 %) and chloralose (1.5 %) mixture solution in a dose of 4 ml/kg intraperitoneally. The animal was tied on a board in supine position, trachea was cannulated and prepared for the record of respiratory resistance on a kymograph as described by Konzett and Rossler (1940). The jugular vein was cannulated by a polyethylene canula to administer the standard and test agents intravenously. Bronchospasm was induced dose dependently by graded doses of histamine (1.25 - 20 µg/kg) intravenously. Fraction F-X was administered in the dose range of 1.25 to 20 mg/kg intravenously. The peak response to drugs was obtained in 1-2 mins. The rest period between two responses was 10-15 mins. to restore the normal respiratory resistance.

Effects of F-X per se or on the submaximal doses of histamine induced bronchospasm was studied. In latter case F-X was given 5 minutes prior to histamine.

2) Compound 48/80 induced mouse hind paw oedema

^{and Bhida} (Ghosh ~~et al.~~, 1980) : Mice of either sex (NIH or Kasauli

strain, 20-25 gms) were used throughout the studies. The hind paw thickness of the animals was measured by "Pocket Thickness Gauge", before and after the injections at appropriate time intervals. In the right paw animals received compound 48/80 dissolved in normal saline and in the left paw an equal volume of saline. Increase in paw thickness was calculated for both paws. The oedema due to compound 48/80 was the difference in increase in thickness, in the compound 48/80 and saline injected paws. The thickness gauge is calibrated in divisions of 0.001 inch each, hence one division was termed as one unit of oedema or thickness.

The dose of compound 48/80 and time for measurement of oedema produced was 0.3 μ g/paw in 0.05 ml saline and 10 seconds respectively (Ghosh and Bhide, 1980).

Fraction F-X (locally, intraperitoneally and intravenously) was tested to cause inhibition of the oedema produced by compound 48/80 in comparison with standard drugs - disodium cromoglycate (local and intraperitoneal) and isoprenaline (local). (Intraperitoneal studies of isoproterenol were not successful due to its fast degradation in the body).

Six animals were used in each group and in control group compound 48/80 was injected in right paw and saline in left paw as described above.

In treated groups, for local studies, the drugs (F-X, DSCG and isoprenaline) in appropriate concentrations (6-24 $\mu\text{g/paw}$, 5-50 $\mu\text{g/paw}$ and 0.1 to 10 $\mu\text{g/paw}$ respectively) were dissolved in compound 48/80 solution in saline such that each animal receives 0.3 μg of compound 48/80 in 0.05 ml with the drug, in the right paw. The drug solutions of identical concentrations in saline without compound 48/80 were used in place of saline for left paws.

For intraperitoneal studies saline solution of F-X and DSCG were injected 30 minutes prior to the experiment in the dose range of 25-100 mg/kg and 0.3-3 mg/kg i.p. respectively.

For intravenous studies, F-X was administered 20 mg/kg i.v. 5 minutes prior to the experiment.

The percentage inhibition in the compound 48/80 oedema formation due to drugs was calculated and significance of each set of experiment was calculated by applying student's 't' test.

Propranolol in a fixed dose of 1 mg/kg was used (given 1/2 hr. prior to experiment) to study its effects per se and in presence of F-X by local and intraperitoneal routes, on the oedema formation, to suggest the mechanism of action.

3) Active paw anaphylaxis in mice (Ghooi and Shide, 1981) : To support the antianaphylactic activity, proved by compound 48/80 oedema model of F-X a new model set up in our laboratory by Ghooi and Shide (1981) was used. For this experiment mice of 57 BL/C strain (either sex 20-25 gms) were used in the groups each of 6 animals.

In the sensitization stage, animals were sensitized with egg albumin 0.25 mg along with aluminium hydroxide gel 6 mg/animal, subcutaneously.

After 8 days the animals were challenged with egg albumin as antigen, locally in the hind paw. In the right paw animals received 0.05 ml egg albumin solution, in saline in appropriate dose while in left paw same volume of normal saline. The paw thickness of both paws before and after injections was measured by Pocket Thickness Gage and oedema produced by antigen-antibody reaction was calculated as described in the previous method.

The optimum dose of egg albumin and time to produce maximal oedema were 10 $\mu\text{g/paw}$ in 0.05 ml saline and 10-15 mins. respectively (Ghool and Bhide, 1981).

The effects of Fraction F-X on the production of oedema by i.p. (25-100 mg/kg) and local (5-20 $\mu\text{g/paw}$) routes and interaction with propranolol (1 mg/kg) were studied as described in the previous experiment, and percentage inhibition of oedema was calculated.

4) Cat blood pressure and respiration : Cats (either sex, 3-4 kg) were anaesthetized by using a solution of urethane (25%) and chloralose solution (1.5%) mixture, in a dose of 4 ml/kg i.p. Trachea was cannulated and connected to a tambour recorder on a kymograph. Carotid artery was cannulated by an arterial canula and blood pressure was recorded parallel to the respiration by connecting the canula through a displacement manometer to the light weight float in the other arm of manometer.

Jugular/femoral vein were cannulated by a polythene canula to inject the standard and test agents intravenously.

Initially, effects of standard drugs as adrenaline (0.1 and 1 $\mu\text{g/kg}$), acetylcholine (1 $\mu\text{g/kg}$), iso-

prenoline ($1 \mu\text{g/kg}$) and histamine ($1 \mu\text{g/kg}$) on both parameters were recorded. F-X, in the dose range of 5-20 mg/kg was injected intravenously, to study its effects per se on both parameters and also on the responses of standard agonists.

Atropine, mepyramine and propranolol were used as cholinergic, histaminic and beta-adrenergic blockers respectively (all 1 mg/kg) and their effects on standard agonist as well as F-X responses were studied.

5) LD₅₀ : F-X was administered to albino mice (either sex, 20-25 gms) in doses ranging from 100 to 1600 mg/kg intraperitoneally with 10 animals/group. The mortality upto 72 hours and any other side or toxic effects were noted and LD₅₀ was calculated by the method of Litdefield.

6) Anti-inflammatory testing : (Inhibition of carrageenan induced rat hind paw oedema - Winter et al., 1962) : The crude petroleum ether (I), chloroform (II) and methanolic (III) extracts in a single dose 400 mg/kg, orally (given 1/2 hr. before experiment), were tested for its anti-inflammatory activity. Oedema was induced in the rat (6 animals/group) (either sex, 120-150 gms), hind paws with 0.1 ml of 1% carrageenan solution in

normal saline solution in planter region. Phenylbutazone (400 mg/kg, oral) was used as standard drug to cause inhibition of oedema and inhibition, if any, in the fractions treated groups was compared with the inhibition due to standard drug. The paw volumes were measured by plethysmometer. Initial readings were taken before injecting carrageenan. Three hours after injecting carrageenan readings of paw volume were again taken and difference in the two readings of each group was the oedema produced. The oedema produced in control group (receiving carrageenan in paw and 1 ml/100 gm saline orally) was considered as 100% oedema and percent inhibition in oedema due to phenylbutazone and fractions, was calculated.

7) Analgesic activity (Tail-Clip Method)

(Soaje-Echaque and Lim, 1962) : Crude petroleum ether (I), chloroform (II) and methanol (IV) extracts (400 mg/kg intraperitoneally, 1/2 hr. before experiment) were studied for their analgesic activity. Mice of either sex, 20-25 gms, NIH strain (10 animals/group) were used. The method used was Tail-Clip method. Standard drug used was aspirin (400 mg/kg) intraperitoneally.

The response to the aspirin and extracts by tail clip method was taken at 15, 30 and 60 minutes intervals. The percent protection with respect to

control group (injected with saline i.p.) of the treated groups was studied.

8) Anticonvulsant activity (Activity against leptazol induced convulsions) (Blanchi and Franceschin, 1954) : Albino mice (either sex, 20-25 gms) were used to induce convulsions, chemically by leptazol 78 mg/kg, intraperitoneally, in groups of 10 animals/group. The crude petroleum ether (I), chloroform (II) and methanol (III) extracts were injected 400 mg/kg intraperitoneally, 1/2 hour before injecting leptazol. The percent protection in the treated groups against leptazol induced convulsions and mortality with respect to control group (injected with saline intraperitoneally) was studied.

PART IV

R E S U L T S

Chapter - 1

PHYTOCHEMISTRY :

A) Results of Extraction :

Method I used for extraction, was successful yielding a semipure fraction, F-X, which was found to be active. The detailed pharmacological results of the fraction are presented in Pharmacological part.

Method II, failed to yield any significantly active compound, as the activity was distributed in different fractions.

The flow diagram of the two methods with the yield and activity is given in the Diagrams 1 and 2 respectively.

B) Results of Chromatography :

The fraction E-VIII and E-VIII failed to get resolved in distinct spots, in any of the chromatographic systems used, in both silica gel and paper chromatography.

C) Results of spot tests :

1) Test for presence of phenols and acids :

a) Tests for phenols :

- 1) Solubility in sodium hydroxide - E-VIII was completely soluble in 1% sodium hydroxide solution ;

DIAGRAM - 1

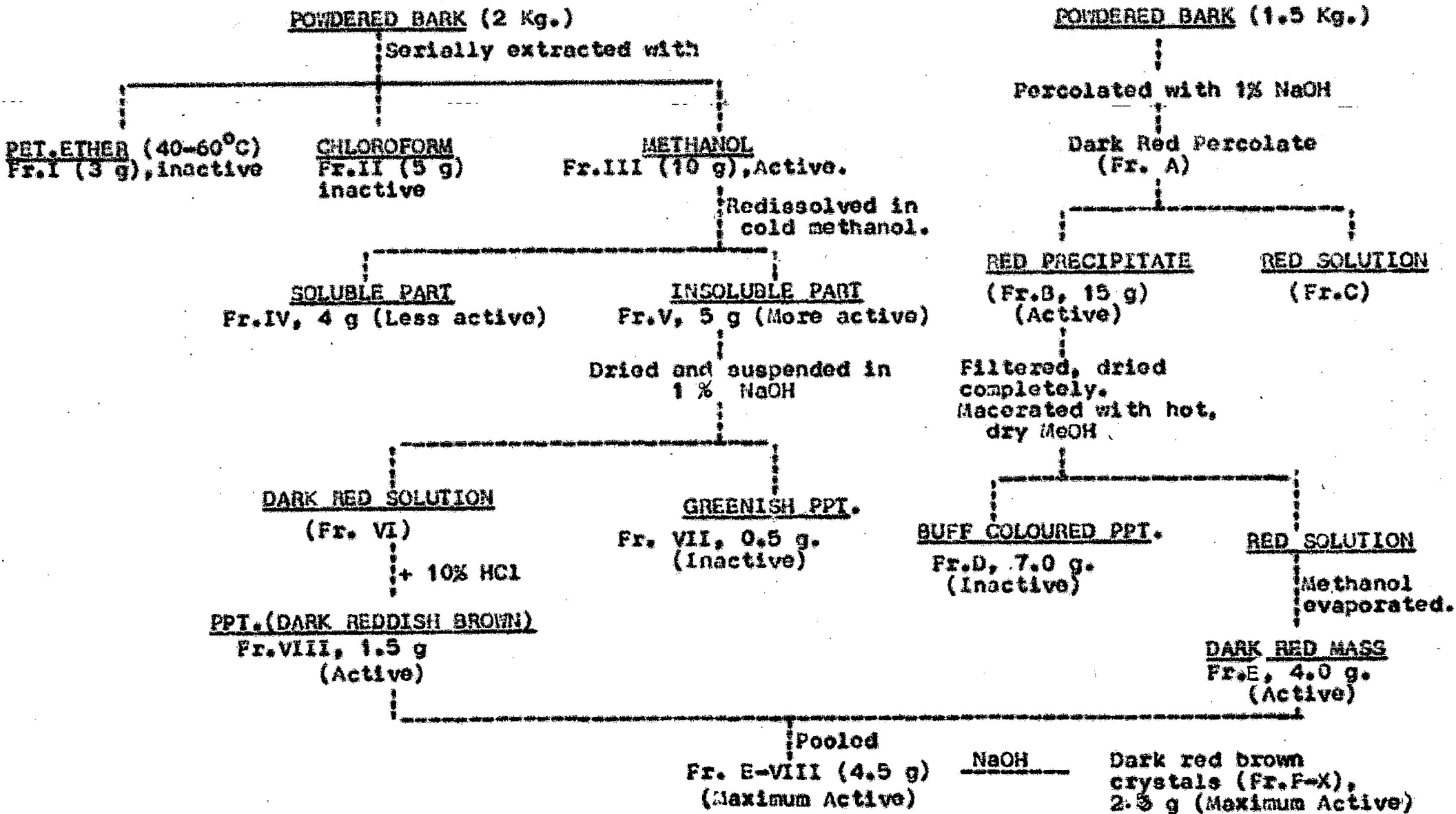
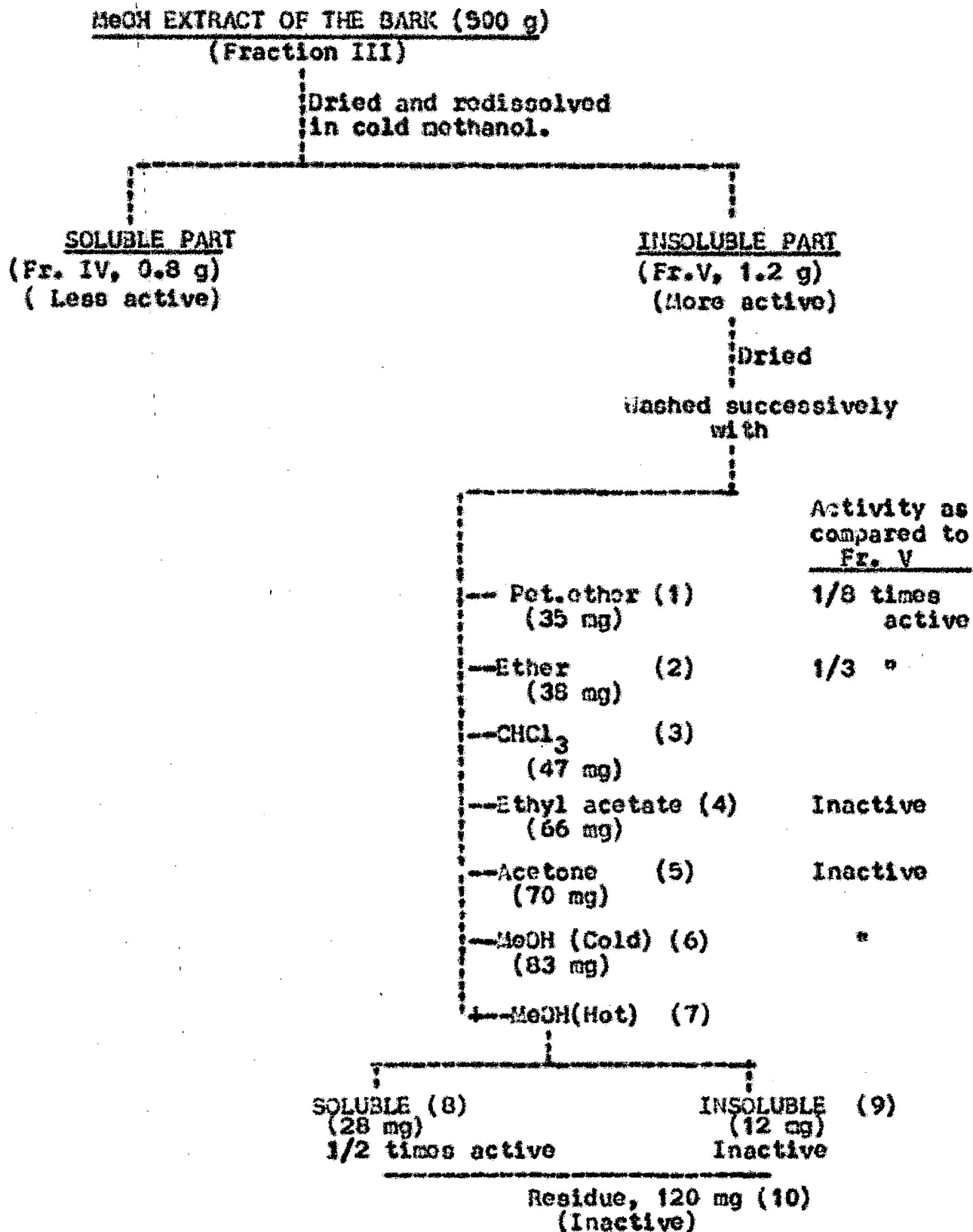


DIAGRAM - 2

ii) E-VIII, on reaction with 1% ferric chloride solution in 0.5N hydrochloric acid showed an ochre colour initially which was turned to greenish yellow solution after 5-10 mins.;

iii) Aluminium chloride solution in alcohol (1%) when sprayed on a spot of E-VIII yielded a orange spot.

b) Tests for acids :

i) Solubility in sodium bicarbonate solution (5%)
The fraction E-VIII was about 50% soluble in the soda bicarb solution.

2) Test for presence of alkaloids :

<u>Reagent</u>	<u>Result .</u>
i) Dragendorff's reagent	Negative
ii) Mayer's reagent	Negative

3) Test for presence of flavanoids :

Colour reactions with -

- i) Aqueous sodium hydroxide solution : A deep roddish brown colour was developed immediately, changing to original yellow colour of the alcoholic solution after 5-6 hours.
- ii) Concentrated sulphuric acid : A dark crimson red colour was developed immediately, which remained unchanged for 8-10 hours.
- iii) Magnesium foil and hydrochloric acid : No colour change was observed. The yellow colour of original alcoholic solution remained unchanged.

4) Test for steroids : Negative.

5) Test for glycosides : Negative.

ISOLATED GUINEA PIG TRACHEAL CHAIN PREPARATION

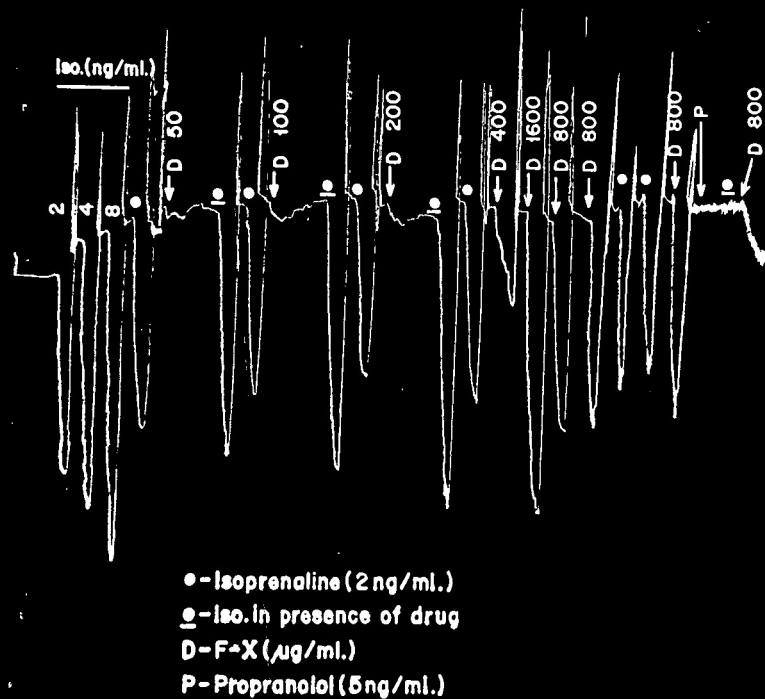


Fig. 2

Chapter - 2

PHARMACOLOGY :

In vitro Methods :

1) Isolated guinea pig tracheal chain :

Isoprenaline, used as reference drug caused a dose-dependent (2 - 8 ng/ml) relaxation of the tracheal smooth muscle. Fraction F-X at lower doses (50-200 μ g/ml) did not produce any per se relaxation but potentiated the submaximal (2ng/ml) dose of isoprenaline to the extent of 20-50 %. At higher doses (400-1600 μ g/ml) F-X produced a dose-dependent relaxation of the smooth muscle. The relaxation produced by 800 μ g/ml of F-X was equivalent to that produced by 2 ng/ml of isoprenaline, thereby showing the large doses of F-X required as compared to isoprenaline.

The specific beta-adrenergic receptor blocker propranolol (5 ng/ml) used as antagonist blocked both isoprenaline as well as F-X responses. Fig. 2 illustrates the results.

2) Isolated guinea pig atria :

Adrenaline in the dose range of 5-80 ng/ml produced dose dependant increase in the force and rate of contractions of the muscle. Fraction F-X in the dose

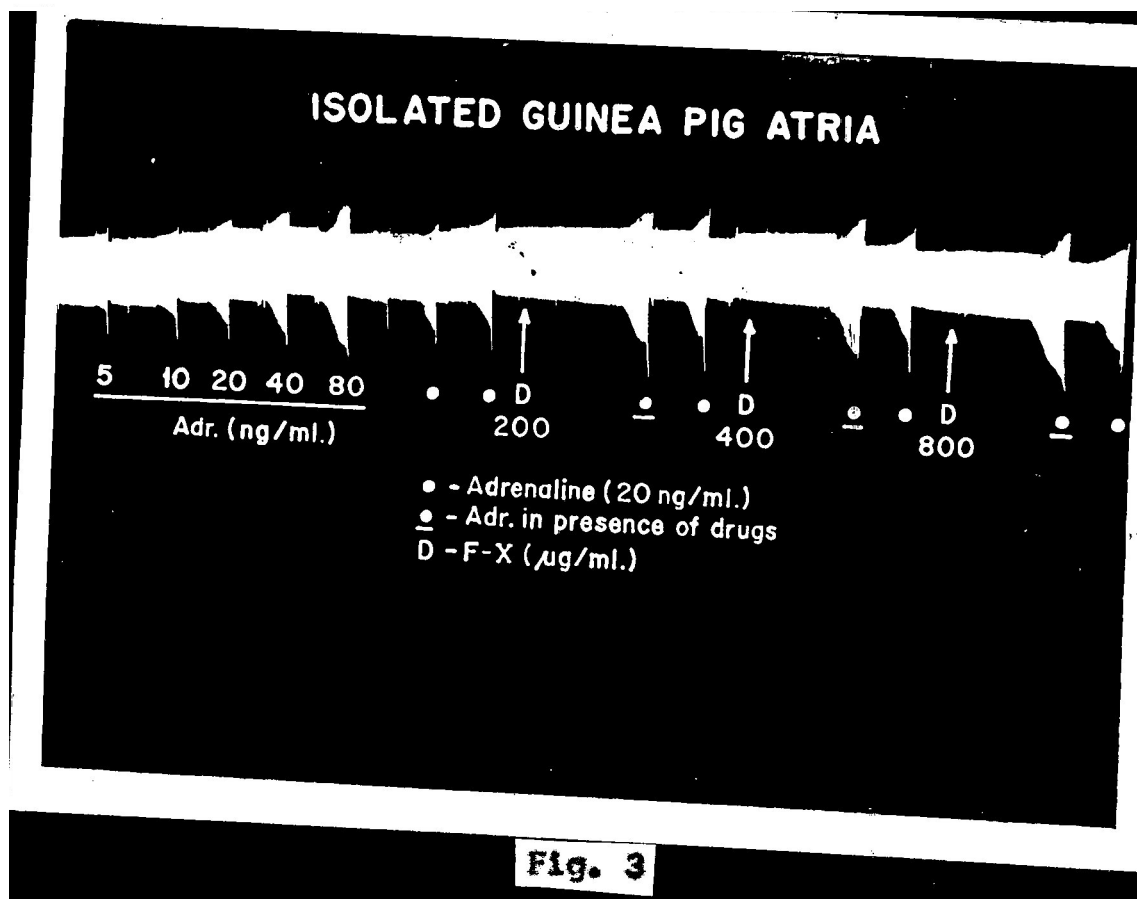
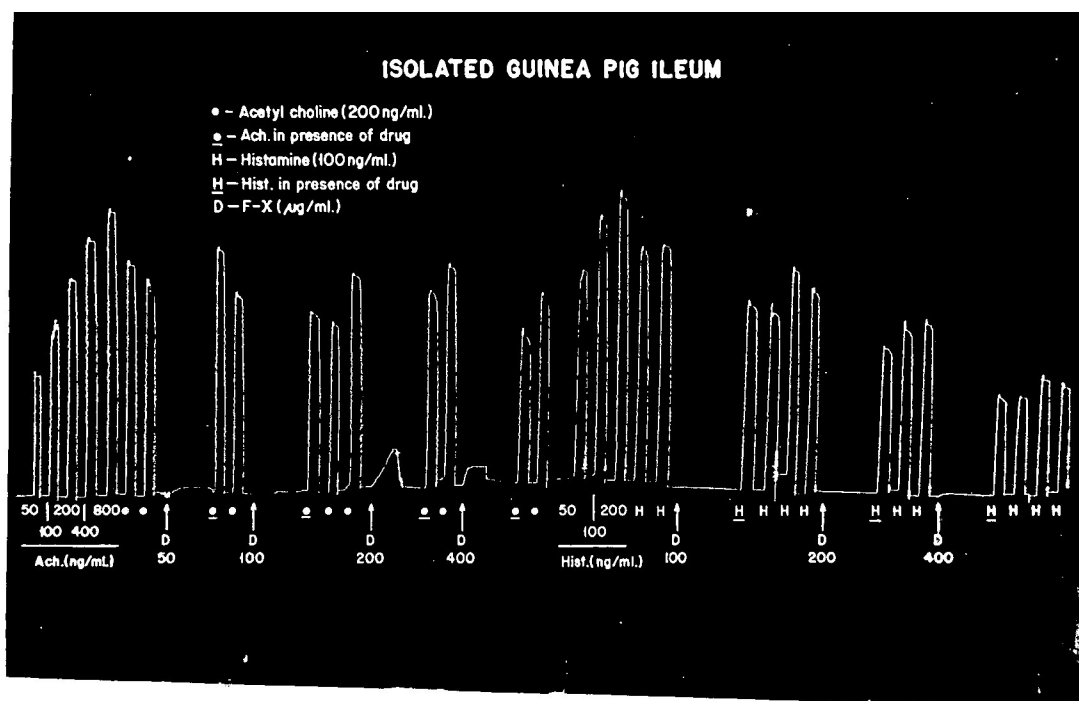


Fig. 3

range 200-800 $\mu\text{g/ml}$ failed to produce any per se effects, but all these doses caused potentiation of submaximal dose (20 ng/ml) of adrenaline (ranging from 20-100 %) dose dependently (Fig. - 3).

3) Isolated guinea pig ileum :

Fraction F-X did not produce any per se effects (contractions or relaxation) of the muscle, but it inhibited the submaximal doses of both the standard spasmogens used - histamine, acetylcholine. Histamine submaximal dose (100 ng/ml) was dose dependently (100-400 $\mu\text{g/ml}$) blocked by F-X to the extent of 20-50%, while the submaximal acetylcholine responses (100 ng/ml) were also blocked by F-X to about 10-20 %, but this inhibition was not dose-dependent (Fig. - 4).

4) Isolated rat uterus :

Carbachol submaximal responses (4 $\mu\text{g/ml}$) were blocked by F-X dose dependently (100-800 $\mu\text{g/ml}$) to the extent of 15-30 %. The oxytocin induced submaximal contractions were also blocked upto 10-15 % by F-X in the same dose range, but this inhibition was not dose-dependent.

5) Isolated frog rectus abdominis :

None of the crude fractions of petroleum ether (I),

AIRWAY RESPIRATORY RESISTANCE IN GUINEA PIG

(KONZETT AND ROSSLER'S METHOD)

● - Histamine (5 μ g/ml.)

● - Hist. in presence of drug

D-F-X (mg/kg)

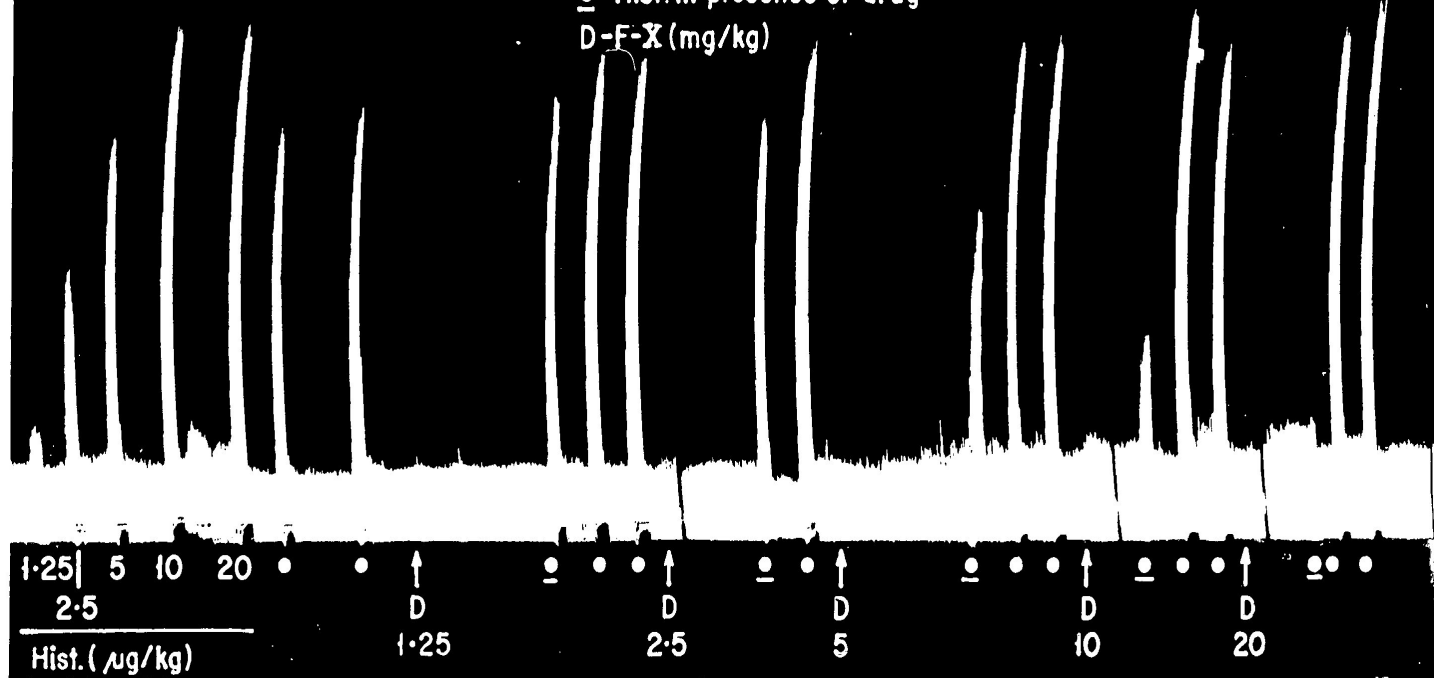


Fig. 5

chloroform (II) and methanol (III) as well as F-X produced any changes (potentiation or blockade) on the submaximal dose of acetylcholine (4 ng/ml), in the dose range of 100-800 μ g/ml for all fractions.

6) Isolated rat phrenic nerve diaphragm :

Neuromuscular conduction of the tissue was not affected by any of the fractions (crude fractions - petroleum ether (I), chloroform (II), methanol (III) and F-X) at the single dose 400 μ g/ml, upto 15 minutes.

In vivo Methods :

1) Airway respiratory resistance in guinea pigs :
(Konzett and Rossler's Method)

Histamine, used as standard drug caused a dose dependent bronchoconstriction in the dose range of 1.25 to 20 μ g/kg. F-X, failed to produce any effect per se in the dose range of 1.25 - 20 mg/kg, intravenously. But the histamine submaximal doses (5 μ g/kg) were blocked by this dose range from 10% to 100% dose dependently. Recovery of the histamine responses was long, showing long duration of action of F-X (Fig. - 5).

2) Compound 48/80 induced mouse hind paw oedema :

F-X (Local intraperitoneal and intravenous), DSCG (local and intraperitoneal) and isoprenaline (local)

inhibited the oedema produced due to compound 48/80, in mice hind paw.

In i.p. administration DSCG was about 100 times active than F-X, but locally F-X was more active than DSCG. Isoprenaline by local route was 10 times more active than F-X. Propranolol 1 mg/kg prevented the inhibition of oedema due to the fraction (in i.p. and local routes). Fig.- 6 shows the dose dependent inhibition of the oedema of F-X, DSCG and isoprenaline, locally. Fig. - 7 shows the inhibition of oedema by F-X by all three routes and prevention of it by propranolol. The detailed results are listed in Tables - 3 to 5.

3) Active paw anaphylaxis :

The oedema produced in sensitized mice hind paw due to antigen (egg albumin) was inhibited by F-X both by local as well as intraperitoneal routes. Propranolol prevented the inhibition of oedema caused by F-X by local route. The detailed results with percentage inhibition dose-dependently by F-X by both routes are illustrated in Fig.- 8 and Table - 6.

EFFECT OF F-X ON COMPOUND 48/80 OEDEMA IN MOUSE PAW

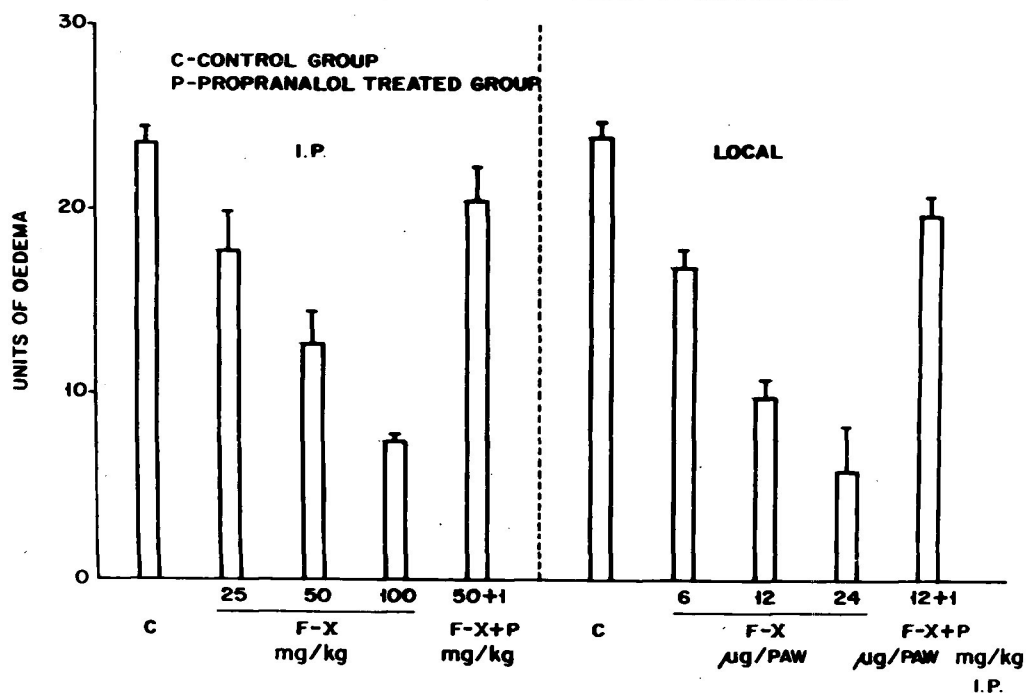


Fig. 7

COMPARATIVE DOSE DEPENDANT INHIBITION OF COMPOUND 48/80 OEDEMA BY F. X, DSCG AND ISOPRENALINE (ROUTE OF DRUGS - LOCAL)

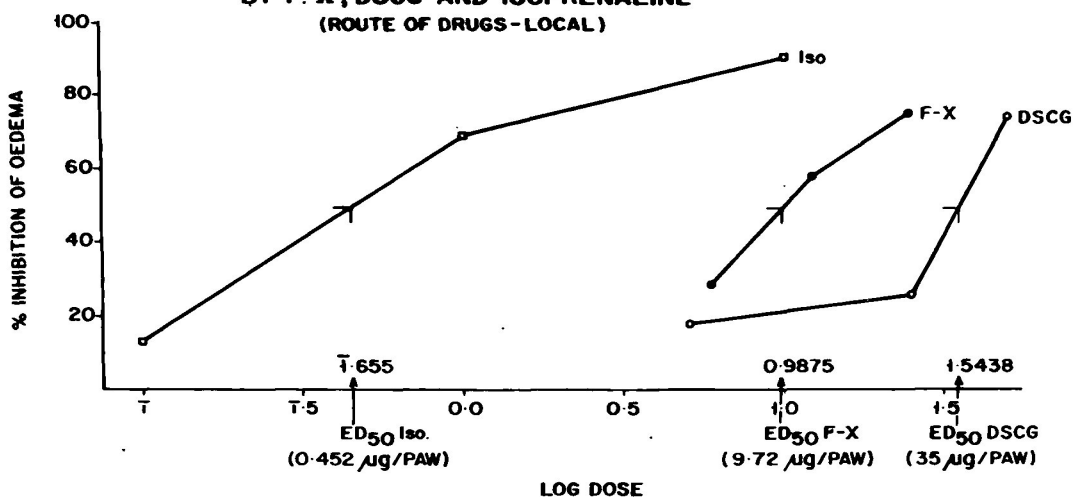


Table - 3 : Effect of F-X on Compound 48/80 oedema.

GROUP	Route	Treatment	Dose (mg/kg)	Mean oedema	$\pm$ S.E.	% Inhibition	Significance by unpaired t-test
1	-	Control	-	23.6	0.94	-	
2.	i.p.	F-X	25	17.83	2.08	25.0	Insignificant
3.	i.p.		50	12.83	1.55	43.64	< 0.05
4.	i.p.		100	7.50	0.35	69.64	< 0.001
1.	-	Control	-	24.00	0.73	-	-
2.	Local	F-X	6 μ g/paw	17.00	0.84	29.2	< 0.01
3.	Local		12 μ g/paw	10.16	0.83	58.66	< 0.001
4.	Local		24 μ g/paw	5.83	1.39	75.70	< 0.001
5.	i.v.		20 mg/kg	11.66	1.33	51.4	< 0.01

Right paw - Compound 48/80 0.3 μ g/paw in 0.05 ml saline;
Left paw - 0.05 ml saline.

**Table- 4 : Effect of DSCG and Isoprenaline on Compound 48/80
Oedema.**

GROUP	Route	Treatment	Dose	Mean oedema	$\pm$ S. E.	% Inhibition	Significance by unpaired t-test
1.		Control	--	20.0	1.80	--	--
2.	i.p.	DSCG	0.3 mg/kg	12.6	0.92	37.00	< 0.10
3.	i.p.	DSCG	1.0 mg/kg	7.5	1.04	62.50	< 0.01
4.	i.p.	DSCG	3.0 mg/kg	4.0	2.12	80.00	< 0.05
1.		Control	--	22.5	0.64	--	--
2.	Local	DSCG	5.0 μ g/paw	18.3	2.56	18.67	Insignificant
3.	Local	DSCG	25.0 μ g/paw	16.5	1.28	26.60	< 0.05
4.	Local	DSCG	50.0 μ g/paw	5.5	0.50	75.5	< 0.001
5.	Local	Isoprena- line	0.1 μ g/paw	19.5	1.47	13.3	Insignificant
6.	Local	"	1.0 μ g/paw	6.85	0.91	69.5	< 0.001
7.	Local	"	10.0 μ g/paw	2.00	0.61	91.1	< 0.001

Right paw - Compound 48/80 0.3 μ g/paw in 0.05 ml saline.

Left paw - 0.05 ml saline.

Table - 5 : Effect of Propranolol.

GROUP	Route	Treatment	Dose	Mean Oedema (in Units) $\pm$ S.E.	% Inhibition	Significance	
1.	--	Control	--	24.00	0.73	--	
2.	Local	F-X	12.0 μ g/paw	10.16	0.83	58.66	< 0.001
3.	Local	F-X + P	12.0 μ g/paw +1.0 mg/kg	19.83	0.83	17.40	a) < 0.10 [*] b) < 0.01
1.	--	Control	--	23.60	0.94	--	
2.	i.p.	F-X	50.0 mg/kg	12.83	1.55	43.64	< 0.05
3.	i.p.	F-X + P	50.0 mg/kg +1.0 mg/kg	20.54	1.95	12.97	a) Insignificant ^a b) < 0.05

P = Dose of propranolol : 1 mg/kg i.p. 1/2 hr before experiment.

R.P. : Compound 48/80 0.3 μ g/paw in 0.05 ml saline.

L.P. : 0.05 ml saline.

* a) As compared to control group; b) As compared to standard treated group.

EFFECT OF F-X ON ACTIVE PAW ANAPHYLAXIS IN MICE

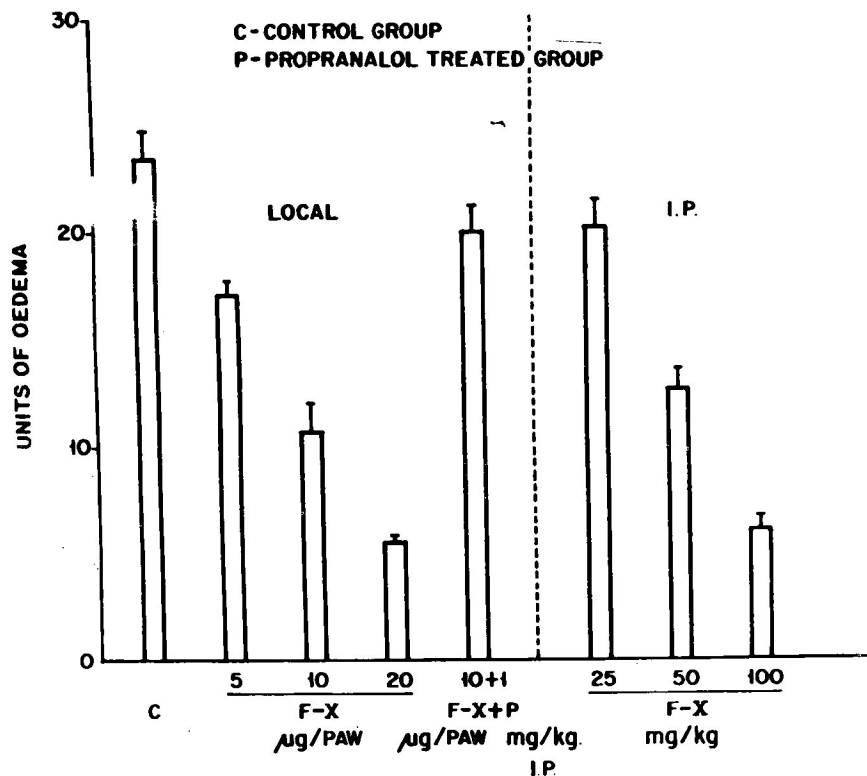


Table-6 : Effect of F-X on active paw anaphylaxis.

GROUP	Route	Treatment	Dose	Mean oedema $\pm$ S.E.		% Inhibition	Significance by unpaired 't' test
1.	-	Control	-	23.6	1.29	-	-
2.	Local	F-X	5 μ g/paw	17.2	0.66	27.12	< 0.005
3.	"	"	10 μ g/paw	10.8	1.24	54.2	< 0.01
4.	"	"	20 μ g/paw	5.6	0.25	76.3	< 0.001
5.	"	F-X + P (i.p.)	10 μ g/paw + 1 mg/kg	20.2	1.15	14.5	a) Significant* b) $< 0.05^*$
6.	i.p.	F-X	25 mg/kg	20.4	1.22	13.6	Insignificant
7.	"	"	50 mg/kg	12.8	0.86	45.8	< 0.01
8.	"	"	100 mg/kg	6.2	0.58	73.8	< 0.001

P = Propranolol.

- * a) As compared to control group;
b) As compared to 3rd group.

4) Cat blood pressure and respiration :

F-X produced a dose-dependent fall in B.P. while the respiration function was also increased dose dependently in the dose range 5-20 mg/kg. The standard agonists responses (ACh, histamine and adrenaline) were not significantly affected by F-X except isoprenaline, which was potentiated to about 20% by the fraction.

The per se effects (viz., fall in blood pressure and bronchodilation) produced by F-X were not abolished by atropine or mepyramine maleate (both 1 mg/kg), but propranolol (1 mg/kg) blocked the bronchodilation caused by F-X completely and fall in blood pressure was blocked to about 30-50%.

5) LD₅₀ :

The highest dose of F-X (1600 mg/kg) did not kill any of the animals in the group upto 72 hours, hence the LD₅₀ could not be calculated. The lower doses (100-400 mg/kg) did not show any side-effects or toxic effects while with higher doses (800-1600 mg/kg) slight CNS stimulation was seen.

6) Antiinflammatory activity :

Crude fractions, petroleum ether (I), chloroform (II) and methanol (III) did not show any significant

activity as against phenylbutazone in rat hind paw carrageenan induced oedema. Results are shown in Table - 7.

Table-7 : Effect of F-X on carrageenan induced rat hind paw oedema.

GROUP	Route	Treatment	Dose (mg/kg)	No. of animals used (Rats)	% Inhibition of oedema.
1.	Oral	Pet.ether(I)	400	6	3
2.	"	Chloroform(II)	400	6	-6
3.	"	Methanol (III)	400	6	11
4.	"	Phenylbutazone	400	6	47

7) Analgesic activity :

Aspirin treated animals produced a 20-50% analgesic activity by tail clip method, as against 0 % in control (saline injected) group. The crude chloroform extract (II) was 10-20% active while other two extracts (petroleum ether-I and methanol-II) failed to show any activity. (Table-8).

Table - 8 : Analgesic effect of F-X by 'Tail Clip Method'.

Group	Route	Treatment	Dose (mg/kg)	No. of mice not responding (in minutes)		
				15	30	60
1.	i.p.	Saline	1 ml/100 g	0/10	0/10	0/10
2.	"	Pet.ether (I)	400	0/10	0/10	0/10
3.	"	Chloroform (II)	400	2/10	1/10	0/10
4.	"	Methanol (III)	400	0/10	0/10	0/10
5.	"	Aspirin	400	5/10	4/10	2/10

8) Anticonvulsant activity :

Crude petroleum ether extract (I) exhibited a 30% protection to leptazol induced convulsions and mortality as against control (saline injected) group having 0% protection. Other two extracts - chloroform (II) and methanol (III) showed a marginal activity.

Table-9 : Effect of F-X on Leptazol induced convulsions in mice.

GROUP	Route	Treatment	Dose (mg/kg)	% Mortality
1.	i.p.	Saline	1 ml/100 g	100
2.	"	Pet.ether (I)	400	70
3.	"	Chloroform (II)	400	90
4.	"	Methanol (III)	400	90

PART V

DISCUSSION AND CONCLUSION

DISCUSSION :

Bronchospasm, pulmonary oedema and hypersecretion of mucous are the root causes of the episodes of dyspnoea, wheezing and cough in asthma. These symptoms are caused mainly due to the release of mediators of anaphylaxis from mast and other cells in pulmonary tissue, by antigen-antibody reaction, in extrinsic asthma. While, a number of agents have been reported to produce relief from attacks, or during an attack reduce its intensity, no curative therapy has yet been described.

By treatment with present drugs against the disease, symptomatic relief is achieved by either inhibiting the mediator release, thereby reducing the intensity of the bronchospasm and oedema, or by minimizing the labour of respiration by broncho-relaxation.

In the former category various antianaphylactic drugs like disodium cromoglycate, corticosteroids, xanthine derivatives, etc. are included which interfere at one of the stages of the anaphylactic reaction to inhibiting the mediator release. In the second category drugs stimulating beta-adrenergic system (like sympatho-

mimetic amines) are included, which mainly cause the bronchorelaxation, but also are helpful to some extent as antianaphylactic agents.

The treatment by any of these drugs is not curative and they provide a symptomatic relief only in a fraction of population on which they are used. Besides, each one of them possesses certain side-effects. For these reasons, therapy is guided by the response and compatibility of individual towards different agents.

Search to develop newer and better drugs is directed towards obtaining an agent which would be more effective than the present drugs. Modification of catecholamine nucleus, for bronchodilator activity or cromone, dioxypurine and steroidal nucleus for anti-anaphylactic activity has been carried out extensively but newer substitutes of these drugs have not changed the situation, dramatically. In the last decade, DSCG and topical steroids like beclomethasone developed from the known active nuclei or anticholinergic agents like methascopalamine, are only few new drugs added in the treatment of the disease.

An alternative approach to the problem of search for new drugs, would be the exploitation of plants

with known activity against asthma and/or allied conditions, and also known to contain similar structure/s which possess antiasthmatic activity. The bark of plant Butea monosperma (Palas) has been employed in Indian system of medicine to prepare a decoction for use in cough and cold (Nadkarni, 1976). From the plant, Bhaskara Rama Murti and Seshadri (1940; 1941); Puri and Seshadri (1953) reported certain derivatives of flavanoids - butin, butein, etc. These flavanoids possess a cromone moiety with a catechol side chain. Hence, it was believed that the plant could be beneficial in the treatment of asthma, and this hypothesis was tested using two types of techniques - one, in which bronchorelaxant effects were studied and other, in which antianaphylactic activity was studied.

One of the difficulties in screening new agents for antiasthmatic activity is the absence of appropriate laboratory model of the disease. The presently used models differ significantly from clinical situations, in both effector organ system and the sensitivity of the species. Church and Gradidge (1980) compared the activity of a series of antianaphylactic agents, in the commonly used laboratory models, with their activity in the clinic. Their results suggest that, techniques like passive cutaneous anaphylaxis, and chopped human

lung anaphylaxis in vitro produces results which are not parallel to clinical results. This is admittedly the most serious drawback of laboratory studies of anti-asthmatic agents.

In the present study, for testing the broncho-dilator activity standard techniques as guinea pig tracheal chain (Castillo and De Boer, 1947); airway respiratory resistance in guinea pigs (Konzett and Rossler, 1940) and cat respiration were used. However, for antianaphylactic screening two recently reported models as "Inhibition of compound 48/80 induced oedema" (Ghooi et al., 1980) and "Active paw anaphylaxis" in mice (Ghooi and Bhido, 1981) were used. Though, the models were new, the authors had reported their validity as standard drugs like DSCG, isoprenaline and theophylline had been proved to be active, comparably with other standard models. In the first model, compound 48/80 is used to produce an anaphylactoid reaction (which is similar to anaphylactic reaction but not produced by antigen-antibody interaction). The anaphylactoid reaction produced by compound 48/80 is much similar to that induced by antigen. Rat peritoneal mast cells (Ranadive and Ruben, 1973; Atkinson et al., 1969), guinea pig skin were used previously to produce this anaphylactoid reaction. The method of Ghooi et al. (1980) using mouse

hind paw is convenient, cheaper and quicker, hence it was selected for antianaphylactic screening. Active paw anaphylaxis in mice was also performed with the same reasoning.

In the phytochemical part of the work, fractions E and VIII isolated by method I were biologically active. Both fractions were almost identical in their properties and hence were pooled together (E-VIII). Various analytical and chemical spot tests were employed for determining the nature of fraction.

The solubility of fraction in 1% sodium hydroxide proved the phenolic nature of the compound. Partial solubility in 5% sodium bicarbonate solution revealed the presence of phenol carboxylic acid structure or presence of acidic ($-\text{COOH}$) compounds. The positive tests given by ferric chloride and aluminium chloride reagents suggested a polyphenolic structure. The colour reactions with sodium hydroxide, conc. sulphuric acid and magnesium/hydrochloric acid indicated the presence of flavanoids. At this stage the fraction was not subjected to further purification or chemical tests, but a sodium salt of the fraction was prepared (F-X) which was used further for detailed pharmacological studies.

The method II for extraction was unsuccessful as the activity of crude extract was distributed in different fractions after sub-fractionation.

In the pharmacological part of the study the bronchodilator effect of F-X was first investigated. In the in vitro guinea pig tracheal chain model, F-X produced marked potentiation of submaximal isoprenaline responses at a lower dose range (50-200 $\mu\text{g/ml}$), without producing bronchorelaxation per se. Potentiation of catecholamine responses is observed in case of a variety of drugs of which phosphodiesterase inhibitors are best known. Isoprenaline is suggested to elevate intracellular cyclic-AMP (cyclic adenosine mono phosphate) levels (Robison et al., 1967) which mediate muscle response. The intracellular cyclic AMP is degraded by phosphodiesterase and inhibitors of this enzyme can potentiate isoprenaline responses by maintaining intracellular cyclic AMP at elevated levels. It is probable that F-X acts in a similar manner. The dose dependent potentiation of adrenaline responses in guinea pig atria, in vitro is also supportive to this conclusion. F-X at higher doses (400-1600 $\mu\text{g/ml}$) produce bronchorelaxant effects in vitro, which were blocked by propranolol, thereby further confirming the weak beta-stimulant activity.

The activity of F-X could not be attributed exclusively to the stimulation of one of the beta-

adrenergic subtypes (beta-1 or beta-2) as it also potentiated the adrenaline responses dose dependently (100-800 $\mu\text{g/ml}$) in isolated guinea pig atria and caused a dose dependent (5-20 mg/kg) fall in cat blood pressure.

On Konzett and Rossler's model, recording airway respiratory resistance in guinea pigs, F-X inhibited histamine induced bronchoconstriction in a dose-dependent manner, at doses ranging from 1.25 mg/kg to 20 mg/kg. F-X on isolated guinea pig ileum demonstrated nonspecific smooth muscle relaxant effects, but it also revealed dose dependent (50-400 $\mu\text{g/ml}$) partial antihistaminic activity. Hence, it was difficult to decide what contribution the antihistaminic effects of F-X have in its bronchodilator activity.

In anaesthetized cat respiration recording, the depth of respiration of animal was increased dose dependently by F-X, and this response was blocked by propranolol. This confirmed the bronchodilator activity of F-X in vivo with involvement of beta-adrenergic system.

Studies on mediator release induced by anaphylactoid reactions indicate that, F-X has potent inhibitory activity on mouse paw oedema induced by compound 48/80, when administered locally (ED_{50} 10 $\mu\text{g/paw}$). But

intraperitoneally the activity was poor ($ED_{50} = 60 \text{ mg/kg}$) suggesting a poor absorption of F-X by i.p. route.

In active paw anaphylaxis similar results were observed. F-X caused a dose dependent inhibition of oedema both locally and intraperitoneally induced by antigen in sensitised mice. As in the above method local ED_{50} of F-X was $9 \mu\text{g/paw}$ while by i.p. route it was 55 mg/kg , thus establishing the premise that i.p. absorption of F-X is not proper.

The activity of F-X as compared to standard anti-asthmatic drugs isoprenaline and DSCG in the anaphylactoid reaction model was twenty times less and three times more respectively, on weight per weight basis by local route (ED_{50} s are as shown below).

<u>Drug</u>	<u>ED_{50} local</u>
F-X	$10 \mu\text{g/paw}$
DSCG	$33 \mu\text{g/paw}$
Isoprenaline	$0.45 \mu\text{g/paw.}$

The beta-adrenergic antagonist, propranolol, almost completely inhibited the actions of F-X, on mediator release, in both models. Such an effect is conducive to the inference that F-X stimulates the beta-adrenergic receptors. Agents acting on the beta-adrenergic receptors have been since long known to inhibit

mediator release (Schild, 1936), and the present study reveals that F-X has a similar effect.

General pharmacological studies of F-X, on other smooth muscles and skeletal muscles failed to reveal any effect worth mentioning. Also it could not produce any lethality upto 1600 mg/kg dose (i.p.) in mice for 72 hrs. and no other significant effect was observed. The crude petroleum ether (I), chloroform (II) and methanol (III) were also devoid of significant anti-inflammatory, analgesic and anticonvulsant activity.

SUMMARY AND CONCLUSION

Butea monosperma is a beta-adrenergic stimulant which potentiates the activity of isoprenaline and adrenaline. It is also a potent antianaphylactic agent when used locally. Both these effects are most prominent in F-X, which is a partially purified fraction from methanolic extract of the bark and is probably rich in flavanoids. While it would be premature to make any further speculations on the chemical nature and activity of the fraction F-X, its potential as an antialsthmatic agent can hardly be doubted. Further studies on the chemistry of the active fraction, and its pharmacology would be fruitful.

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APPENDIX -I

Physiological salt solutions used

Constitution per litre		Tyrode	Kreb's	Feigen's	DeJalon	Frog Ringer
10% Solutions	Sodium chloride (g)	8.0	6.9	9.0	9.0	9.0
	Glucose (g)	1.0	2.0	1.0	0.5	1.0
	Sodium bicarbonate (g)	1.0	1.0	0.6	0.5	0.5
	Calcium chloride (ml)	2.0	2.8	6.2	0.3	2.4
	Potassium chloride (ml)	2.0	4.0	4.2	4.2	4.2
	Sodium dihydrogen phosphate (ml)	0.5	--	--	--	--
	Pot. dihydrogen phosphate (ml)	--	1.6	--	--	--
	Magnesium sulphate (ml)	--	1.4	--	--	--
	Magnesium chloride (ml)	0.1	--	--	--	--

SYNOPSIS

SYNOPSIS

INTRODUCTION

Bronchial asthma has been defined as a condition of subjects with wide spread narrowing of bronchial airways, which changes its severity over short periods of time, either spontaneously or under treatment and it is not due to the cardiovascular disease.¹ Falling under this definition are the two types of asthma - the intrinsic type and the extrinsic type. The psychogenic factors and the complement system play a pivotal role in intrinsic type, while the extrinsic type is mainly due to allergic responses causing localized anaphylaxis in the bronchial airways. The local anaphylaxis in this situation is manifested as bronchoconstriction, endothelial oedema or collapse of small bronchioles, together leading to inadequate alveolar exchange of gases.

Anaphylactic reactions are a result of the interaction between target cell bound cytotoxic antibody (Ig E) and the specific antigen. The interaction causes an increase in the permeability of the cell membrane and a release of chemical mediators, that produce effects

such as smooth muscle contraction, vasodilatation and formation of oedema. The conventional bronchodilator drugs prevent bronchospasm by dilating the bronchus, specifically or non-specifically. The present philosophy is to use the drugs which can selectively inhibit the formation and/or release of mediators.

It is estimated that almost 10% of the world population suffers or is likely to suffer from episodes of dyspnoea, during its life time.² The magnitude of the problem emphasizes the need for newer and better drugs to combat this condition. The conventional drugs in use for asthmatic and allergic conditions are -

- 1) beta receptor stimulating catecholamines and non-catecholamines; 2) theophylline group of compounds; 3) corticosteroids and 4) the most recent disodium cromoglycate.

Though all these drugs are used in present therapy of bronchial asthma and allergic manifestations, each one of them possesses certain drawbacks. Thus, almost all catecholamines and noncatecholamines used in asthma possess central nervous system stimulating effects and cardiovascular effects. However, some recently developed drugs in this category like salbutamol, soterenol, etc. show least of these side effects at their therapeutic doses.

The major drawback in theophylline group of compounds is the central nervous system stimulation.

The major objection to the corticosteroid therapy is their hindrance with the normal hormonal function, which in some cases can prove to be fatal.

In the case of disodium cromoglycate it is stated that 50% of the patients do not yield any benefit to the treatment.² Also it has no effects on acute attacks and hence it is mainly used as a prophylactic agent.

For all these limitations of the conventional drugs, attempts are made in the laboratories to develop newer and safer drugs, either from plant sources or from realm of synthetic chemistry. Quite a few plants have yielded compounds which are of use, though limited, in bronchial asthma. Outstanding amongst these are theophylline (Thea sinensis), ephedrine (Ephedra sinica, E. equisetina, etc.) and the recently studied vasicinone (Adhatoda vasaka).³

In Ayurveda the bark and juice of the plant, Butea monosperma Roxb, family leguminosae (Palas, Palaash), has been described to be of value in alleviating broncho-

spasm. A decoction of the bark is useful in catarrh, cough and cold.⁴ The plant is reported to contain chalcones like Butoin, Butin and their glycosides, kinno tannins and gallic acid, etc.⁵

In the preliminary studies the bark of the plant exhibited a bronchodilator effect. Hence we were interested in further investigation on this plant with a specific reference to its bronchodilator and antiasthmatic activity, if any, in the laboratory models and to establish the mechanism of action.

The work was divided in two parts -

- A) Phytochemical work : Isolation and purification of biologically active extract.
- B) Pharmacological work : To establish the antiasthmatic and/or antiallergic activity of this extract using different in vitro and in vivo techniques and to study its mechanism of action.

A) PHYTOCHEMICAL WORK :

As the extract of the bark in preliminary studies showed a bronchodilator effect, the same was used further to isolate the active fraction. At every stage of separation the bronchodilatory and probable antiallergic activity was assessed following the methods of :-

- 1) Isolated guinea pig tracheal chain preparation.⁷
- 2) Compound 48/80 induced hind paw oedema in mice.⁸

The fraction showing positive activity on these two models was used for further purification.

The bark was air-dried in shade, powdered (1 kg) and extracted serially with petroleum ether (40-60°C) (dry wt. 1.5 g), chloroform (dry wt. 2.5 g), and methanol (dry wt 5.0 g), according to increasing polarity. It was found that methanolic extract was active while pet. ether and chloroform extracts were inactive in both models. Hence methanol extract was chosen for further purification.

The methanolic extract (5 g) was completely dried and redissolved in cold methanol. It was found that the extract could not be redissolved fully in cold methanol. The soluble (2 g) and insoluble (3 g) parts were separated and screened for their activity. The insoluble part was found to be active.

The insoluble extract (3 g) was extracted with 1% sodium hydroxide solution. A dark red solution of total acids and phenols from the extract was obtained with a small amount of greenish residue. This residue was discarded as it was inactive. To the sodium hydroxide

solution 10% hydrochloric acid was added till a dark brown coloured mass precipitated. This precipitate was filtered, dried and collected (1.5 g).

A part of this precipitate was used to form sodium salt by using 0.1N sodium hydroxide solution. To the precipitate, sodium hydroxide was added gradually until the precipitate was just dissolved (pH 7.0). This solution was evaporated to dryness at 50°C when the sodium salt of total acids and phenols crystallized out. This salt fraction was further used for detailed pharmacological studies.

B) PHARMACOLOGICAL WORK :

To establish the pharmacological activity of the sodium salt of the purified fraction, various in vitro and in vivo laboratory techniques were used as mentioned below :-

I) In vitro techniques :

1) Isolated guinea pig tracheal chain preparation⁶ : The tracheal smooth muscle contracts and relaxes transversely. Hence trachea was cut in small rings and rejoined to form a chain, so as to increase the length of the muscle. The tissue was mounted in a

10 ml bath containing Kreb's solution gassed with carbogen. Relaxation of the muscle was recorded on a kymograph using isotonic frontal writing lever. Isoprenaline and propranolol were used as standard agonist and antagonist respectively. Effect of the fraction per se and on isoprenaline responses was studied.

2) Isolated guinea pig atria : The isolated atria was mounted in a 10 ml bath with Feigins solution with oxygen as aeration and contractions were recorded on a kymograph using Starling heart lever. Adrenaline was used as standard agonist to cause an increase in the force and frequency of contraction. Effect of the fraction per se and on the adrenalinic responses was studied.

3) Isolated rat uterus : Single horn of the isolated rat uterus was mounted in a 10 ml bath in DeJalon's solution using carbogen as aeration. Isotonic lever was used to record contractions on kymograph. Oxytocin and carbachol were used as standard agonists to induce contractions and effect of fraction per se and on responses induced by standard drugs was studied.

4) Isolated guinea pig ileum : One inch piece of the distal end of the guinea pig ileum was mounted in a 10 ml bath containing Tyrode solution gassed with

air. The contractions were recorded on a kymograph using isotonic lever. Acetylcholine and histamine were used as standard agonists. Effect of fraction per se and on the responses induced by standard agonists was studied.

II) In vivo techniques :

1) Airway respiratory resistance in guinea pigs (Konzette and Rossler's method) : The guinea pig (350-400 g) of either sex was anaesthetized with chloralose (25%) and urethane (1.5%) mixture in the dose of 4 ml/kg i.p. The trachea was cannulated and the respiratory resistance was recorded on a kymograph by connecting the tracheal cannula to a displacement manometer. The respiratory resistance was increased by i.v. injection of histamine used as standard agonist and effect of fraction per se on the respiratory resistance and histamine induced resistance was studied.

2) Inhibition of the compound 48/80 induced mouse hind paw oedema : Compound 48/80 in the dose of 0.3 μ g in 0.05 ml saline was injected in the right hind paw of the animal to induce oedema by histamine release. In the left paw 0.05 ml saline was injected as control. The right and left paw volumes were measured at 10 min. of injection using pocket thickness gauge. The

difference between right and left paw volumes was considered as standard oedema produced. The effect of the fraction on the inhibition of oedema was studied by using various routes of administration of drugs such as local, i.v. and i.p.

3) Cat blood pressure and respiration : The cat (either sex, 3-4 kg) was anaesthetized with chloralose (25%) and urethane (1.5%) mixture in a dose of 4 ml/kg i.p. The trachea was cannulated and expired air volume was recorded on a kymograph with a tambour recorder. Blood pressure was recorded simultaneously by cannulating carotid artery through a displacement manometer. The drugs were injected intravenously through jugular vein. The standard agonists used were acetylcholine, histamine, adrenaline and isoprenaline. Standard antagonists used were atropine, propranolol and mepyramine. The effect of the fraction per se and on the standard agonist was studied.

RESULTS :

1) In vitro studies :

1) Isolated guinea pig tracheal chain preparation : On this preparation the fraction and isoprenaline caused dose dependent relaxation

(200-1600 $\mu\text{g/ml}$ and 1-8 ng/ml respectively). Propranolol completely blocked the responses due to the fraction and isoprenaline.

2) Isolated guinea pig atria : The fraction did not show any effect per se but potentiated adrenaline responses.

3) Isolated rat uterus : No significant effect was observed on either oxytocin or carbachol induced contractions in this preparations.

4) Isolated guinea pig ileum : The fraction partially inhibited the responses induced by acetylcholine and histamine but this inhibition was not dose dependent.

II) In vivo studies :

1) Konzett and Rossler's method : The fraction exhibited dose dependent (1.25 - 10 mg/kg) inhibition of the histamine induced bronchoconstriction in anaesthetized guinea pigs.

2) Inhibition of compound 48/80 induced mouse hind paw oedema : The fraction caused dose dependent (4-16 $\mu\text{g/paw}$) inhibition of 48/80 induced hind paw oedema in mice. The pretreatment with propranolol

prevented this inhibition of oedema due to the fraction. The inhibition of oedema due to the fraction was also observed when administered i.v. and i.p.

3) Cat blood pressure and respiration : The fraction produced dose dependent fall in blood pressure (5-20 mg/kg) which was not blocked by atropine, propranolol or mepyramine. In respiration record slight increase in the expired air volume was seen which may be due to the bronchodilatation and this effect was not blocked by above antagonists.

CONCLUSION :

The active fraction isolated from methanolic extract of the bark of Butea monosperma Roxb (Palas) mainly contains acidic and phenolic compounds. The fraction exhibited bronchodilator activity in guinea pig tracheal chain preparation and airway respiratory resistance in guinea pigs (Konzett and Rossler's method). It also stimulated respiration in anaesthetized cat and produced stimulatory effect in isolated guinea pig atria. Propranolol completely blocked the responses induced by the fraction in tracheal smooth muscle preparation.

The fraction also inhibited the compound 48/80 induced mouse hind paw oedema which was prevented by

propranolol. From this it can be inferred that fraction possessed an antiallergic activity by inhibiting histamine release.

The data reveals that the fraction possesses bronchodilatory, cardiac stimulating and antiallergic activity which may be mediated through beta adrenergic system.

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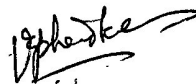
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STATEMENT UNDER UNIVERSITY RULES Nos. 0.412 & 0.413.

As required by the University Rule Nos. 0.412 and 0.413 I state that the work presented in this thesis is of original in nature and so far, to the best of my knowledge, has not been submitted for any degree of this or any other University.

Whenever reference has been made to the work of others, it has been clearly indicated along with the source of information in the "Bibliography".



(Uday R. Phadke)

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