CHAPTER 2

REVIEW OF LITERATURE

2.1 TOBACCO SMOKING

Tobacco (Nicotiana tabacum) smoking is a very common habit among millions of people worldwide. According to the World Health Organization (WHO), tobacco-attributable mortality is projected to increase from 1.5 million deaths in 1990 to 3.0 million annually by 2020, in India [13].

2.1.1 History of tobacco use

The cultivation and collection history of tobacco is about 8000 years old. Tobacco was first time used by the people of the pre-Columbian Americans [17]. Tobacco was introduced in India by Portuguese traders in about 16th century. Few years back the risk of spreading tuberculosis was recognized as spitting of smokeless tobacco, so the trend of consuming tobacco has majorly shifted towards cigarette smoking [17]. In India, the British and American Tobacco Companies spread the tobacco trade by establishing the Imperial Tobacco Company in India, now known as Indian Tobacco Company (ITC) Ltd. [64]. Besides cigarette smoking, the year 1920 is marked for other means of tobacco usage like; cigars and pipes etc. Tobacco products (smoked and smokeless) are also being used in several other forms also, which includes cigarettes, cigarillos, bidis, kereteks and chuttas etc. [17].

2.1.2 Bidi smoking - prevalence and habit

“Beedi” or “Bidi” or “Beeri” is a form of hand-rolled cigarette in which sun-dried temburni or tendu leaf (Diospyros melanoxylon) is rolled into a conical shape together with flaked tobacco and secured with a cotton thread (Figure 1.1) [65]. The bidis are known as the “poor man’s cigarettes” as, they are cheaper than cigarettes and are perhaps the cheapest tobacco smoking product in the world. A bidi is 4 - 8 cm long having a diameter at the closed end is approx 0.6 - 0.8 cm and 0.7 - 0.9 cm
at the smoking end [17]. Bidi smoking is quite common in rural India specifically, in low income groups as; it is the cheapest tobacco smoking product available all over the world [15]. Analysis of data available for cigarettes and bidis manufacturing in India (year 1910 to 2010) suggests an increase in bidi production over the former (Figure 2.1) [13].

![Figure 2.1: The Data collection from the Ministry of Agriculture, Government of India about estimated bidis and cigarettes manufactured in India during a span of 100 years [13].](image)

### 2.1.3 Chemistry of tobacco smoke

Tobacco smoke contains approx >4800 toxic compounds which have been categorized into carcinogens, co-carcinogens, tumorogenic compounds as well as, mutagens [66]. The tobacco smoke generated during burning of “tobacco products” can be divided into:

- **Mainstream smoke** – tobacco smoke released by the burning of a cigarette at the mouth end
- **Side stream smoke** – tobacco smoke produced by the burning of a cigarette at cone side

Besides this, people present around an active smoker are also exposed to tobacco smoke (Second hand tobacco smoke - passive smoking), which is a mixture of side stream smoke and exhaled mainstream smoke. It has been reported that mainstream tobacco smoke from cigarettes contain around 3996 complex constituents [67]. The amount of nicotine, alkaloids, carcinogens, and other harmful compounds varies in different sections of a burning bidi. Moreover, the country of its origin also affects its chemical composition due to their geographic location.
specificity, methods of production of tobacco and methods of production of tobacco products. The Indian and Italian cigarettes are found to possess extremely high levels of tobacco specific carcinogens [58,000 ng/g N’-nitrosonornicotine (NNN) and 10,745 ng/cigarette 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)] [17]. It has also been reported that the levels of NNN and NNK in tobacco of bidi range from 6,200 - 12,000 ng/g and 400 - 1,400 ng/g tobacco, respectively [17]. It is important to note that the amount of nicotine has been found to be more in bidi in comparison to the commercially available filter-tipped and untipped American and Indian brands of cigarette [66]. The leaf wrapper of a bidi allows lesser air dilution of the mainstream smoke or volatile species like carbon monoxide (CO), due to its poor porosity and lack of filter ventilation holes [66, 15]. Also, bidis have low combustibility and are to be re-lit several times as they self-extinguish. Hence, bidi smokers inhale the smoke more deeply and this leads to delivery of greater amount of CO, tar, nicotine and other tobacco smoke components as compared to cigarette smokers [18]. Smokers face an increased rate of nicotine absorption in plasma as smoking bypasses the hepatic metabolism system and nicotine gets absorbed and distributes by blood directly from the lungs throughout the body [65].

Tobacco smoke can be divided into two phases: tar and gas-phase smoke. Both phases contain abundant number of free radicals and carcinogens. The free radicals are identified in tobacco smoke include reactive oxygen and nitrogen species (ROS & RNS) like superoxide (’O2\textsuperscript{-}), hydroxyl (’OH) and peroxyl (’RO2\textsuperscript{-}), and RNS like nitric oxide (NO\textsuperscript{-}), nitrogen dioxide (NOO\textsuperscript{-}) and peroxynitrite (ONOO\textsuperscript{-}) etc. [68] table 2.1. Besides these free radicals, it also contains several harmful chemical compounds like volatile hydrocarbons, polynuclear aromatic hydrocarbons, nitrohydrocarbons, heterocyclic hydrocarbons, N-heterocyclic amines, aromatic amines, aldehydes, 10-N-nitrosamines, phenols and various other organic compounds (Table 2.1). It also contains various inorganic compounds like nickel, lead and cadmium, etc., also [17].
Table 2.1: List of ROS and RNS

<table>
<thead>
<tr>
<th>Type</th>
<th>ROS</th>
<th>RNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free radicals</td>
<td>Superoxide (O$_2^-$), alkoxyl (RO$^\cdot$),</td>
<td>Nitric oxide (NO$^\cdot$), nitrogen dioxide (NOO$^\cdot$)</td>
</tr>
<tr>
<td></td>
<td>hydroxyl (OH$^\cdot$), hydroperoxyl (HOO$^\cdot$), peroxy (RO$_2^\cdot$)</td>
<td></td>
</tr>
<tr>
<td>Non-radicals</td>
<td>Hydrogen peroxide (H$_2$O$_2$), ozone (O$_3$), hydrochlorous acid (HOCl),</td>
<td>Peroxynitrite (ONO'O'), dinitrogen trioxide (N$_2$O$_3$), nitrous oxide (HNO$_2$), alkyl peroxynitrites (RONOO), Other tar radicals are Quinine/hydroquinone radical (Q/QH$_2$ radical)</td>
</tr>
</tbody>
</table>

As, discussed above The toxic compounds and free radicals of tobacco smoke, gets absorbed into the blood stream from the respiratory tract from where, they reach to various organs of the body like: heart, pancreas, liver and kidney etc. thus, cause toxicity in those organs/tissues [68]. On the other hand, the particles from the particulate fraction of the smoke get adhered to lung tissue and causes local and peripheral injury due to the release of adhered toxins and oxidants from the particles, for hours till days, resulting in progressive cellular injury and mucus membrane destruction.

2.2 Respiratory disorders

Respiratory disorders are some of the most common medical conditions in the world including India. Tens of millions of people suffer from lung disease in the U.S every year [6]. Tuberculosis, pneumonia, chronic obstructive pulmonary disease (COPD), bronchitis; asthma and cystic fibrosis etc., are the most common respiratory disorders. Air pollution, smoking, infections, and genetic predisposition etc., are majorly responsible for most of these pathological conditions [21]. Among these tobacco smoking has been identified one of the major cause for respiratory disorders. Tobacco smoke contains huge amount of toxic components and present in both the nonvolatile and volatile phases. These all components are known to be carcinogenic and affect whole respiratory system.
2.2.1 Types of respiratory disorders

According to causing factors of the disease, respiratory disorders are classified as - infectious and non-infectious respiratory disorders [69].

**A. Infectious respiratory disorders:** Tuberculosis, pneumonia, bronchitis and emphysema are the most common infectious respiratory disorders caused by infections like: *Mycobacterium tuberculosis*, *Streptococcus pneumonia*, *Bordetella pertussis* and *Chlamydophila pneumoniae*, respectively [70].

**B. Non-Infectious respiratory disorders**

COPD, Asthma, bronchitis, cystic fibrosis, tumors etc., are most common non infectious respiratory disorders. Asthma and COPD are inflammatory lung diseases which are known to be caused by exposure to environmental stressors such as pollution, smoking, UV radiation and dust etc., [71]. Asthma is a chronic inflammatory disorder of the airways characterized by episodes of reversible breathing problems due to airway narrowing and obstruction. These episodes can range in severity from mild to life threatening [72]. COPD is a preventable and treatable disease which is characterized by airflow limitation that is not fully reversible [73]. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases (typically from exposure to cigarette smoke) [74].

2.2.2 Epidemiology of respiratory disorders due to tobacco smoking

Tobacco smoke has remarkable negative impacts for those who smoke it. It is a major cause of inflammatory lung disorders and lung cancer both men and women [75]. In the United States, more than 400,000 deaths are expected yearly due to high addiction potential of tobacco [76].

Prevalence of respiratory disorders with such an increasing rate has made it one of the world’s commonest diseases and, acquiring the position of one of the biggest public-health problems accounting for atleast half-a-billion cases [77, 78]. Several other leading causes of respiratory disorders include: stressful life, polluted environment, physical inactiveness with unhealthy eating habits, etc. [79].
Epidemiological and clinical studies have shown that smokers are significantly more likely to develop diseases like emphysema, asthma and smoker’s cough etc [80]. Smoking cigarettes causes numerous changes in the lungs and airways such as, mucus producing cells in the lungs and airways grow in size and number, thereby increase the amount of mucus produced and function of cilia is hampered, as a result, the lungs and airways get irritated and inflamed [81]. They become narrow and the airflow in the lungs reduces. When lung tissues are destroyed, the number of air spaces and blood vessels in the lungs also decrease. Hence, the smoker’s lungs become more susceptible to allergies, and infections [82]. Prolonged exposure to tobacco smoke can even lead to lung cancer [83]. The main mechanism reported to be involved in most of the diseases and disorders is oxidative stress [84]. Tobacco smoke is known to increase oxidative stress in most of the organs in our body and have also been reported to be involved in respiratory and other disorders [85].

2.3 Respiratory disorders and oxidative state the system

Respiratory diseases like, Asthma and COPD are inflammatory lung diseases that are characterized by systemic and chronic localized inflammation [86]. Reactive species like ROS and RNS are normally required by the cells for cell signalling mechanism and a balance between these reactive species and antioxidants is maintained by the cells (“redox homeostasis”). An imbalance between the two is defined as “oxidative stress” and it is one of the most common factors reported to cause inflammation [85]. These outnumbered radicals can interact with the cellular molecules (proteins, carbohydrates, DNA, RNA, mtDNA, and membrane lipids etc.) and cell organelles (membrane, mitochondria and nucleus etc.) hampering their normal metabolic activities and can lead to various types of metabolic dysfunctions and cell death (Figure 2.2) [86].

Exogeniously, tobacco smoke can act as a trigger and can enhance a rise in ROS/RNS produced if, exposed to a biological system [87]. An increase in reactive species has been linked to activation of various signaling pathways like: mitogen-activated protein kinase (MAPK) and also the increase in activation of transcription factors including NF-κB and AP-1 [88] which have been implicated to enhance the
processes like: inflammation, apoptosis, proliferation, transformation and differentiation (Figure 2.2) [86].

Endogenously, ROS are majorly produced in mitochondria during electron transport system (respiration). The role of ROS produced in phagocytes is proposed to be to recruit other immune cells to sites of inflammation which causes clearance of the pathogen and also a huge cell and tissue damage. In chronic inflammatory conditions, including asthma and chronic obstructive pulmonary disease (COPD), a large amount of cell and tissue damage is found to be associated with this inflammation [89].

Figure 2.2: Diagramatic representation of events going on inside a normal cell after smoking and the oxidants and antioxidants involvement during this condition.

2.4 Redox state of cells in a smoker

2.4.1 Role of oxidants

As, discussed before, tobacco smoke disturbs the redox state of the exposed biological system. Tobacco itself contains huge amount of free radicals/ROS and RNS which are delivered to the exposed system, directly. Besides this, various components of tobacco smoke induce their formation also in the exposed biological system. Normally, endogenous defense mechanisms play a key role in combating the harmful effects of ROS but, in a smoker, oxidants level exceeds over the antioxidants and impairs the physiological functions [90]. Subsequent induction of oxidative stress initiates toxic effects in cells and tissues, which has been implicated.
in several human lung diseases like asthma and COPD etc. (Figure 2.3) [91, 92, 93].

2.4.2 Role of Antioxidants

ROS are also generated naturally in biological system but, they are maintained at normal levels by the endogenous enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidise (GPx) system and non-enzymatic antioxidative mechanisms.

The reactive species like \(^\cdot\)O\(_2\) radicals thus generated, get scavenged by the antioxidant enzyme like Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase etc. Superoxide dismutase is a prime antioxidant that scavenges the excess superoxide radicals in the cells. The activity of this enzyme (SOD) has been found to have variations in the results obtained by various scientists (decreased or increased or showed no change) in several respiratory study models [94, 95]. Superoxide can be dismutated to H\(_2\)O\(_2\) by superoxide dismutase. H\(_2\)O\(_2\) is more stable and lipid soluble, so can go through cell membranes having a longer half life than \(^\cdot\)O\(_2\), which get further scavenged by Catalase and Glutathione peroxidase to form water. The subsequent section is a detailed discussion on these pathways and associated cellular pathways that get affected due to this oxidant-antioxidant imbalance (Figure 2.3).
Figure 2.3: Various pathways leading to generation of highly reactive free radicals (highlighted in purple) in a smoker. Oxygen gets converted to superoxide via activated enzymatic and non-enzymatic pathways. It is then dismutated to H$_2$O$_2$ by Superoxide dismutase (SOD) and, finally converted to H$_2$O by Catalase and Glutathione peroxidase (GSH-Px). H$_2$O$_2$ can also get converted to hydroxyl radical (•OH) through Fenton reaction. GSH is regenerated by Glutathione reductase. Also, •O$_2$ reacts rapidly with NO• to form a highly reactive ONOO•. Besides generation of free radicals inside the cells, these radicals are also presenting in/produced from external agents like cigarette smoke, etc. [85].

2.5 Oxidative stress and cell death

Cell death is controlled machinery present in the cells, which is required for their development, immune regulation and homeostasis. It can be divided into two major classes: Apoptosis and Necrosis. The nature of cell death signal determines whether a cell is to undergo either of these death mechanisms. These two distinct mechanisms of cell death differ from each other on morphological, biochemical and cytological grounds.

Experimentally Necrosis is characterized by nuclear morphological changes appearance of smear pattern during DNA gel electrophoresis [96, 97], swelling of the cells and plasma membrane disruption.

Apoptosis is an energy dependent programmed cell death mechanism. It involves death receptor ligation mediated release of mitochondrial apoptotic mediators that lead to activation of caspases (cysteine-dependent aspartate-directed proteases) in cytosol. This causes proteolytic cleavage of several cellular targets that eventually lead to cell death [98]. A series of biochemical events lead to morphological changes in the cells like membrane blabbing, cell shrinkage, nuclear
fragmentation, chromatin condensation and DNA fragmentation (~200 bp fragments), with maintenance of intact plasma membrane [96, 99]. It has been reported that free radicals affect the covalent modifications to the histone proteins and DNA thus, altering gene expression, and so triggering apoptosis [100]. The apoptotic mechanism of cell death occurs via two pathways (Figure 2.4):

a. The intrinsic/mitochondrial pathway
b. The extrinsic/cell death receptor activated pathway

![Figure 2.4: Apoptotic pathways leading to cell death. The extrinsic pathway involves binding of ligand to death receptor which causes formation of death-inducing signalling complex (DISC) and activation of procaspase 8. Caspase 8 directly activates procaspase 3 which further cleaves proteins responsible for cell viability and brings about cell death. Caspase 8 links the extrinsic and intrinsic pathways through cleavage of Bid. This puts mitochondria at the centre point of controlling cell death by apoptosis [100].](image)

During OS inside a cell, the increase in ROS leads to increase in expression of the cytokine TNF-α which further aggravates OS [101]. Both the extrinsic and intrinsic pathways have meeting points through caspases 3 which, when initiates the apoptotic cascade and activates other effector caspases in lane, causing cell death. Caspase 8 is reported to cleave Bid (BH3 interacting death domain), a Bcl2 family member protein that causes oligomerization and insertion of Bax (Bcl2 associated X protein) or Bak (other Bcl2 family members) into the outer mitochondrial membrane (OMM). This event joins both the extrinsic and intrinsic pathways, which is followed by OMM permeabilization and release of proteins like cytochrome c from mitochondrial intermembrane space. Entry of cytochrome c into cytosol leads to its binding with Apaf-1 and ATP, thereby activating procaspase 9.
to caspases 9. Caspase 9 further activates the effector caspase 3 and, this finally leads to apoptosis [99].

So, increased ROS in mitochondria mainly affects inner membrane phosphoprotein cardiolipin [102]. This leads to opening of mitochondrial permeability transition pore which leads to release of Bax-α, and cytochrome c. Kuo et al (2005) proposed two main mechanisms on cigarette smoke-induced apoptosis in rat models [103]. The first one relies on the activation of p38/JNK-Jun-FasL signaling. The second is mediated by p53 stabilization, increased Bax/Bcl-2 ratio, and release of cytochrome c. These mechanisms alter the function of mitochondria and nucleus in smoker’s lung cells [104]. All these events trigger apoptosis leading finally to cell death [104].

2.6 Oxidative stress and inflammation

Inflammation is a defensive mechanism to eliminate the causative stimuli as well as, to initiate the healing process of the tissue. But long-lasting inflammation or chronic inflammation, leads to a progressive shift in inflammatory cells to the tissue. As, mentioned before, reactive species are found to be majorly responsible to initiate inflammation and main mechanism involved in this process is through the activation of transcription factors (NF-κB and AP-1 etc.) and other signal transduction pathways (MAP kinases and PI-3K), which enhance gene expression of pro-inflammatory mediators (TNF-α, IL-6 and GM-CSF) causing inflammatory diseases (Figure 2.5) [105].
Figure 2.5: The signalling pathways in pulmonary inflammation. Various stimuli, such as TS, mitogens, and bacterial infection etc., can induce inflammation via the NADPH oxidase/ROS, PKCs, PI3K/Akt, and MAPKs pathways and the transcription factors, such as NF-κB and AP-1 in lung resident cells [105].

2.7 Therapeutic options for inflammatory respiratory diseases

In the current time, there are many therapeutic options available for the treatment of chronic inflammatory diseases like asthmatic and COPD. Three main lines of anti-inflammatory treatment available for asthma: 1) inhaled glucocorticoids, which have multiple mechanisms of action; 2) cysteinyL-LT inhibitors and 3) β2-agonists, which are not only, very effective bronchodilators, but also exert a mild anti-inflammatory action. Most of these synthetic drugs can effectively alleviate oxidative and inflammatory injury but, most of the time they are associated with many adverse side effects (ex: increased rates of pneumonia, shakiness, heart palpitations, dry mouth and urinary tract symptoms etc) which limit acceptance among the population [106]. As, natural products are considered to be comparatively safer, economic and are also commonly available, the acceptance for the herbal product is more in this way.

2.8 Respiratory disorders and use of natural products

Use of natural products is prevalent in India since the time of Ayurveda. Ayurveda - “the science of life”, is originated in India in the pre-vedic period and dates back to 1000 years B.C. [107]. India has about 45,000 plant species and
several thousands have been claimed to possess medicinal properties to treat different diseases including respiratory ailments [108].

World Health Organization (WHO) estimates that, up to 80 percent of people still refer mainly traditional remedies such as: herb(s) and their formulation(s), for the treatment of various diseases [106]. Numerous medicinal plants and their secondary metabolites have been evaluated for their successful uses to treat various inflammatory and respiratory disorders.

2.8.1 Medicinal plants and antioxidants

Most of the medicinal plants carry antioxidant properties as, they contain several types of endogenous compounds like: polyphenols, flavonoids, terpenoid and steroid etc [100]. Beside these compounds they also have, antioxidants like: vitamins A, C and E, carotenoids, glutathione (GSH), and coenzyme Q10 (CoQ10), which play an important role their medicinal value [109].

2.8.2 Antioxidants and Respiratory disorders

Antioxidants like vitamins C and E and black tea have been reported to have beneficial effects in tobacco-induced respiratory disorders in rats. These agents have been shown to lower the LDH, Malondialdehyde level and GSH-Px activity and increase the CAT and SOD activities, significantly [110, 111].

Few of the medicinal plants (used to treat respiratory disorders), their doses along with their uses in a particular ailment, are listed in Table 2.2. Among these medicinal plants Adhatoda vasica has been reported to be used extensively for various respiratory disorders. It has been reported to have significant antioxidant and anti-inflammatory potential [112].

Several medicinal plants have been explored for their various cellular targets of action in stressed conditions. Although, Adhatoda vasica has also been extensively used to treat respiratory and other ailments yet, the exact mechanism of action of this plant is not known. In the present study, we specifically tried to investigate the role of this plant in the tobacco smoke induced stressed conditions.
Table 2.2: Medicinal plants used in Respiratory diseases [113]

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Plant</th>
<th>Dose</th>
<th>Ailments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aegle marmelos</em> (L.)</td>
<td>1 kg of leaves boiled in 4 L of water with one coconut fruit pulp and made into decoction. 200 ml of this decoction is taken orally twice a day for 3-4 weeks.</td>
<td>Asthma</td>
</tr>
<tr>
<td>2.</td>
<td><em>Capparis zeylanica</em> L.</td>
<td>Leaf juice is taken orally with a cup of fresh goat’s milk for 2-3 days.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cardiospermum halicacabum</em> L.</td>
<td>A handful of fresh leaves is kept in fire and the smoke is inhaled, daily once for 1-2 days.</td>
<td>Cough</td>
</tr>
<tr>
<td>4.</td>
<td><em>Allium sativum</em></td>
<td>500 mg/kg body wt. ethanolic bulb extract</td>
<td>Asthma, cough etc.</td>
</tr>
<tr>
<td>5.</td>
<td><em>Berberis aristata</em></td>
<td>Methanolic extract.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>6.</td>
<td><em>C.sinensis</em> (Green tea)</td>
<td>450 mg/kg aqueous leaf extract.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>7.</td>
<td><em>Carissa carandas</em> L.</td>
<td>Roots are ground in lemon juice and half-teaspoonful of root juice taken orally with water, daily once for 2-3 weeks.</td>
<td>Asthma</td>
</tr>
<tr>
<td>8.</td>
<td><em>Clematis gouriana</em> Roxb.</td>
<td>Flowers crushed with fruits of <em>Piper longum</em> L. and made into pills and dried. One pill taken orally with honey, in an empty stomach for 4-5 weeks.</td>
<td>Asthma</td>
</tr>
<tr>
<td>9.</td>
<td><em>Embelia ribes</em> Burm.f.</td>
<td>A handful of roots ground in lemon juice or buttermilk and 1-3 teaspoonful of juice orally with sugar/jaggery, daily twice for 2 days.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>10.</td>
<td><em>Panax ginseng</em></td>
<td>250 and 500 mg/kg body wt. root extract.</td>
<td>Bronchitis, Cough and cold</td>
</tr>
<tr>
<td>11.</td>
<td><em>Momordica charantia</em></td>
<td>13.33 g/kg body wt. aqueous pulp extract.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>12.</td>
<td><em>Murraya koenigii</em></td>
<td>600 mg/kg aqueous leaf extract; 200 mg/kg methanolic leaf extract.</td>
<td>Bronchitis, Cough and cold</td>
</tr>
<tr>
<td>13.</td>
<td><em>Glycyrrhiza glabra</em> L.</td>
<td>One teaspoonful of root powder orally with a cup of water, twice a day for 10 days.</td>
<td>Asthma</td>
</tr>
<tr>
<td>14.</td>
<td><em>Ruta graveolens</em> L.</td>
<td>2-3 teaspoonfuls of leaf juice orally, daily early in the morning for 1-2 days.</td>
<td>Cough and cold</td>
</tr>
<tr>
<td>15.</td>
<td><em>Adhatoda vasica</em> (Justicia adhatoda) L.</td>
<td>One teaspoonful of root paste + garlic + pepper paste + human breast milk early morning for 5-7 days. Pills of crushed leaves, and 1 pill taken orally, daily thrice for 2-3 days.</td>
<td>Bronchitis, Cough and cold etc</td>
</tr>
</tbody>
</table>

The plant - *Adhatoda vasica*
*Adhatoda vasica* (AV) is a perennial, evergreen shrub. The leaves are lanceolate to ovate - lanceolate, base-tapering, petiolate; which appear in 10 - 30 cm long and 3 - 10 cm broad. The flowers of AV are pink, white or purple [55].

*Adhatoda vasica* is one of those valuable plants which have been used against a broad array of diseases especially for respiratory ailments like; dry cough, asthma, bronchitis, common cold, sore throat, smoker’s cough and others like: menstrual disorders, eye infections, skin diseases, bleeding, diarrhea and abortifacient etc [114].

### 2.8.1 Taxonomical nomenclature

According to the Global Biodiversity Information Facility (GBIF), the taxonomical nomenclature of *Adhatoda vasica* is as follows [115]:

- **Kingdom**: Plantae
- **Division**: Angiosperms
- **Class**: Dicotyledonae
- **Subclass**: Gamopetalae
- **Order**: Personales
- **Family**: Acanthacea
- **Genus**: Adhatoda
- **Species**: vasica

### 2.8.2 Geographical distribution

It is widely distributed in India, Malayan region, Punjab in the North, Bengal and Manipur in the South-East area. It is also seen in Sri Lanka, Lower and Upper Myanmar, Southern China, Laos and the Malay - Peninsular and Indonesian Archipelago, found up to 1350 m height [53].

### 2.8.3 Traditional uses

Traditionally, the juice and decoction of the leaves and roots of *Adhatoda vasica* are used in the respiratory disorders like asthma, bronchodialatory, chronic cough and breathlessness [116]. The plant has been included in *The WHO Manual The Use of The Traditional Medicine in Primary Health Care*, to profit health workers in South - East Asia for their surrounding flora [117]. *Adhatoda vasica* has been
used to cure constipation, rheumatism, gout, cough, asthma, tuberculosis, bleeding disorders, ear aches and pus etc., [53, 118, 119].

2.8.4 Parts used

Parts of the plant used to treat the diseases and disorders are: leaves, fruits, flowers and roots. In the present study leaf powder is used.

2.8.5 Vernacular names

Each plant possesses its own vernacular name according to region of distribution. *Adhatoda vasica* carries the following vernacular names:

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanskrit</td>
<td>Vasa</td>
</tr>
<tr>
<td>Hindi</td>
<td>Arusa, Bakas, Adusa, Rusa</td>
</tr>
<tr>
<td>English</td>
<td>Vasaka</td>
</tr>
<tr>
<td>Kannada</td>
<td>Adusoge, Kurchigida, Pavate, Bansa</td>
</tr>
<tr>
<td>Bengali</td>
<td>Basak, Bakas</td>
</tr>
<tr>
<td>Gujrati</td>
<td>Aradusi, Adulso</td>
</tr>
<tr>
<td>Marathi</td>
<td>Adulsa</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Adalodakam</td>
</tr>
<tr>
<td>Kashmiri</td>
<td>Bahekar, Baikar, Bansuth, Babading</td>
</tr>
<tr>
<td>Punjabi</td>
<td>Bhekkar</td>
</tr>
<tr>
<td>Oriya</td>
<td>Arusa, Basung</td>
</tr>
<tr>
<td>Telugu</td>
<td>Addasaramu</td>
</tr>
<tr>
<td>Konkani</td>
<td>Adusogae</td>
</tr>
<tr>
<td>Tamil</td>
<td>Adhatodai, Pavettai</td>
</tr>
</tbody>
</table>

2.8.6 Chemical constituents

The plant has been reported to contain various groups of phytocompounds like alkaloids, phytosterols, polyphenolics, glycosides, proteins, fats and vitamin C etc. [119]. Major chemical constituents of this herb are shown in table 2.3:
### Table 2.3: Chemical constituents of *Adhatoda vasica*

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Chemical constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>Vasicine, 1,2,3,9-tetrahydro-5-methoxy-pyrrol[2,1-b]quinazoline-3-ol, 1,2,3,9-tetrahydro-pyrrol[2,1-b]-quinazolin-9-one-3R-hydroxy-3(2’-dimethylamino phenyl desmethoxy-aniflorine), 7-methoxy-3R-hydroxy-1,2,3,9-tetrahydro-pyrrol[2,1-b]-quinazolin-9-one(7-methoxy-vascione), 7-methoxyvascione hydrate, 5-methoxyvascione, l-vasicol</td>
</tr>
<tr>
<td>FL</td>
<td>Kaempferol, 3-methylheptanone, quercetin, rhamnosylvitexin,</td>
</tr>
<tr>
<td>RT</td>
<td>Tritriacontane, sitosterol-β-D-glucoside, O-ethyl-α-D-galactoside</td>
</tr>
<tr>
<td>LF/FL</td>
<td>Violanthin, vitexin, rhamnosylvitexin, 2''O-xylosylvitexin</td>
</tr>
<tr>
<td>LF/RT</td>
<td>Vasicinol, maiontone, l-vasicinone</td>
</tr>
<tr>
<td>LF/FL/RT</td>
<td>Vasicine, vasicinolone, vasicinone</td>
</tr>
</tbody>
</table>

FL = Flower, LF = Leaf, RT = Root.

Among the several phytoconstituents of this plant, Vasicinone, Vasicinolone and Vasicine have been reported to be the main active phytoconstituents [120]. The leaves, roots and flowers of this plant have been reported to possess significant antioxidant and anti-inflammatory potential and also posses the main phytoconstituents of AV. Among these major phytoconstituents, Vasicine has been widely explored for its antioxidant and anti-inflammatory properties but, its mechanism of action under various pathological conditions has been investigated to a very low extent. So far, we have not found any study with this plant or Vasicine investigating their benefits in tobacco smoke caused toxicities/diseases/disorders. Hence, besides AV, Vasicine was also taken up in our study to elucidate its mode of action.

#### 2.9.7 *Adhatoda vasica* for respiratory disorders

This section reviews the research reports available till date on *Adhtaoda vasica* linked to various disorders including respiratory disorders.

*In vitro, in vivo and clinical studies with plant/plant extract*
AV has been proven to have anti-oxidant and anti-inflammatory properties. Different extracts of this plant have been prepared by various scientists to explore its efficacy and mechanism of action, for example: the methanolic extract of *Adhatoda vasica* was evaluated for anti-inflammatory activity by Chakrabarty and Brantner in 2001. The alkaloid fraction of the methanolic extract of *Adhatoda vasica* has shown potent anti-inflammatory activity at a dose of 50 μg/pellet in hen’s egg chorioallantoic membrane [120].

The ethanolic extract of *Adhatoda vasica* has been demonstrated to have antioxidant and anti-inflammatory activity against carrageenan and formalin-induced inflammation in albino rats [121]. These antioxidant and anti-inflammatory activities of ethanolic extract had been linked to the presence of various flavonoids and other polyphenolic moieties present [121].

Ethanolic extract (800 mg/kg body weight, 6-30 d post irradiation intervals) has also been investigated for its effects on the hematological parameters in Swiss albino mice. After exposing these mice to radiation (8.0 Gy) showed sickness like anorexia, lethargy, ruffled hairs and diarrhea and such animals died within 25 days of post-irradiation. But, when animals were pre-treated with AV leaf extract, it has shown a significant increase in GSH content and decrease in LPO level in blood [122].

These reports depicts that *Adhatoda vasica* plant have potent antioxidant and anti-inflammatory activities and specifically, ethanolic extract is majorly found to have these properties. As, in smokers the respiratory diseases are found to be linked with free radicals (reactive oxygen and nitrogen species), it was postulated that, this plant may also be useful in the conditions where tobacco smoke is the major cause for initiating or deteriorating the conditions of respiratory disorders.

### 2.9.8 Vasicine for respiratory disorders

**Sources**

Vasicine/Peganine (Figure 2.6) is a quinazoline type alkaloid compound (C_{11}H_{12}N_{2}O) mainly obtained from the plant *Adhatoda vasica* (zeylanica)/*Justicia adhatoda* (Acanthaceae). Vasicine molecule was first isolated in 1924 and most of
the work on this molecule was done between 1960s - 1980s. Vasicine was isolated from different plants like *Sida cordifolia*, *Galega battiscombei*, and *Galega lindblomi*. [123, 124, 125]

![Figure 2.6: Structure of Vasicine.](image)

**In vitro, in vivo studies and clinical studies with Vasicine and its derivatives**

Vasicine has been examined for its beneficial effects in respiratory diseases like, asthma etc. The bronchodilatory activity of Vasicine has been suggested to work through respiratory sensors and peripheral receptors [126]. Its derivatives (3-lithiodeoxyvasicine and biaryl) bronchodilator activity was evaluated on contracted trachea and constricted tracheo-bronchial tree. It showed relaxant effect on intestinal smooth muscle too, which was more potent than theophylline but, less to that of salbutamol on dose basis [127].

Antioxidant and anti-inflammatory properties of Vasicine are also been examined against ascorbate and ferrous sulphate induced toxicity in rats and it has been found to improve the antioxidant status of the system by increasing the enzyme activity of superoxide dismutase, catalase, glutathione peroxidase and “reduced glutathione” content [128].

Derivatives of Vasicine are also worked upon to investigate their bronchodilatory and antitussive effects. One of those derivatives is Bisolvon/bromhexine (N-cyclo-N-methyl-(2-amino-3,5-dibromo-benzyl) amine hydrochloride) has been reported to possess mucus liquefying/expectorant activity [129, 130, 131]. One more study was conducted for bromhexine with 30 patients (20 d, 8 mg, thrice a day) suffering from variety of respiratory complaints [132]. It was found to have major changes in the viscosity and acid mucopolysaccharide (AMPS) structure in the mucus of infected and uninfected patients.
Bruce and Kumar (1968) further observed AMPS fiber system to be disintegrated and disorganized with a concomitant fall in viscosity. A total of 100 human patients were given this drug for trial and were reported to respond well for the ease for expectoration of less viscous sputum [133]. On the contrary, Langlands (1970) did not observe any significant change after this treatment [134]. In this study, Bromhexine was compared with a placebo in a double-blind clinical trial in patients with exacerbations of chronic bronchitis who had mucoid sputum. Treatment with Bromhexine 8 mg, three times a day or with identical placebo tablets was continued for 14 days. There was no significant effect on the characteristics of the sputum, improvement in ventilatory capacity, or clinical advantage in patients on Bromhexine. Similarly, a report with usage of a higher dose of Bromhexine was published in “British Journal of Disease Chest” in 1973 [135]. It states that 48 mg Bromhexine dosage daily for 2-3 weeks brought about an indistinguishable effect with the placebo tablets with respect to stickiness of sputum, difficulty of expectoration or time taken to clear the chest in the morning.

Ambroxol, a widely used secretolytic agent developed from Vasicine, is found to inhibit IgE-dependent mediator secretion from human mast cells and basophils, which are the main effector cells of allergic inflammation. This compound was found to be more potent than Vasicine in attenuating basophil IL-4 and IL-13 secretions, respectively. It also reduced IgE-dependent p38 MAPK phosphorylation in basophils [136].

2.9.9 Other pharmacological activities of Adhatoda vasica and Vasicine

- Hepatoprotective activity

AV was proposed to possess hepatoprotective property mediated through its antioxidant property. Pandit et al. (2004) [58] examined the antioxidant potential of Adhatoda vasica against hepatotoxicity induced by carbon tetrachloride in rats. It was found that AV (100 and 200 mg/kg) augmented the activity of hepatoprotective enzymes. Oral doses of 50 - 100 mg/kg of AV leaf extract have also been found to possess significant hepatoprotective effect against D-galactosamine-induced liver damage in rats [137].
• **Anti ulcer activity**

   It is also reported to be the anti-ulcer activity of *Adhatoda vasica* leaves using two ulcer models (1) Ethanol-induced, and (2) Pylorus ligation plus aspirin-induced models [50]. *Adhatoda vasica* leaf powder showed a considerable degree of anti-ulcer activity in experimental rats when compared with a control. 80% recovery was observed in case of ethanol-induced ulcer in rats in comparison to the control rats and 41% in case of aspirin induced peptic ulcer [138].

• **Muscle stimulant activity**

   Madappa et al. (1989) investigated the effect of Vasicine (1 and 10 μg/ml) on mammary gland, uterus, guinea pig ileum and guinea pig tracheal muscle [139]. Vasicine had shown stimulatory effects on rat/guinea pig uterus and tracheal muscle. Its effects were compared with (+) INPEA (nifenolol) it was found that, effect of (+) INPEA are selective for uterine tissue. Vasicine potentiated the action of oxytocin in isolated rat mammary strip preparation. It also showed smooth muscle stimulant activity and is thus used for bronchodilation and abortion [139].

• **Anti-helminthic activity**

   There was one *in vitro* study conducted by Al-Shaibani et al. (2008) to investigate the ovicidal and larvicidal effect of AV extracts against gastrointestinal nematodes of sheep [140]. The water and ethanol extracts of the plant at concentration range of 25-50 mg/ml were found to have ovicidal and larvicidal properties in a dose dependent manner and ethanolic extract of AV was found to be more effective. The maximum ED$_{50}$ values of the AV extract was found to be 18.2 mg/ml (for both the extracts) against the eggs of *Chaberita ovina*. Whereas, the lowest values of 12.59 and 11.48 mg/ml were obtained for ethanolic and aqueous extracts, respectively against the eggs of *O. circumcinta*. Similarly, the highest ED$_{50}$ values were 19.5 and 18.62 mg/ml against *O. columbianum* larvae and lowest ED$_{50}$ values were 15 and 12.88 mg/ml against the *H. contotus* larvae for aqueous and ethanolic extracts, respectively.
• **Anticestodal activity**

Naga tribes have been using the AV plant parts for curing intestinal worm infections, indigenously. The methanolic extract of AV was used to study the anticestodal activity against *Hymenolepis diminuta* infection in rats. A high dose of 800 mg/kg was shown to be significantly effective as, the number of eggs per gram of faces was lowered to 79.6%. The percentage recovery from the eggs was also reduced to 16.6%, in comparison to the control. As, the two major alkaloids were present in the methanolic extract of the leaves are Vasicine and Vasicinone, the observed anticestodal activity was attributed to these two alkaloids [145].

• **Anti-bacterial activity**

A number of semi-synthetic derivatives of Vasicine like bromhexine, benzylamines, and ambroxol, which are usually employed as mucolytics can also exert a pH-dependent growth-suppressive action on *Mycobacterium tuberculosis* (MT). Reportedly, these derivatives accumulate in the macrophages and assist in expelling mucus, containing the bacteria. Also, these compounds enhance the secretion of lysozymes and rifampicin (if given along) in the lung tissue thus proving these to be a useful adjunctive in the management of tuberculosis [141]. Karthikeyan et al. (2009) studied the antibacterial activity of different leaf extracts (i.e., aqueous, ethanolic and petroleum ether) of *Adhatoda vasica* were against a number of bacterial strain (e.g., *B. subtilis, S. epidermidis, S. aureus, K. pneumonia, E. faecalis, P. vulgaris, E. coli, P aeroginosa, and C. albicans*). Mainly, the ethanolic and petroleum ether extracts emerged as powerful anti-bacterial against many strains [142]. The antibacterial activity of these extracts was proposed to be a result of individual or combined effect of active constituents like alkaloids, phenols, tannins and reducing sugars etc. including its main active principle Vasicine [143].

• **Genoprotective role**

Jahangir *et al.* (2006) investigated the radioprotective/genoprotective potential of *A. vasica*. They observed that AV effectively mitigated the oxidative stress induced genotoxicity caused by cadmium chloride. This protective effect of AV was attributed to Vasicine and Vasicinone, the two main alkaloids in the AVE [144].
In one more *in vitro* study methanolic extract of AV was shown to protect 50% of the radiation induce chromosomal aberrations, in human peripheral lymphocytes. Both low and high (50 and 100 mg/kg body wt) doses were added to the peripheral cell culture, of the patients undergoing radiotherapy. Both the doses were able to increase the number of normal metaphases (in total 200 metaphases studied in control and experimental sets), increasing to 179 and 184 (for low and high doses, respectively) in comparison to 118, in control.

In another study, effect of ethanolic extract of AV (800 mg/kg body weight) was studied in Swiss albino mice and the effects were studied on hematological changes triggered by irradiation. Mice exposed to radiation (8.0 Gy) suffered from anorexia, lethargy, ruffled hairs and diarrhea and died within 25 days post-irradiation. Conversely, pre-treatment with AV leaf extract improved survival by 81.25% till 30th day. A robust decrease in blood “reduced glutathione” (GSH) content and increase in lipid peroxidation (LPO) level was observed in control animals (radiation alone). Further, animals pretreated with AV leaf extract significantly increase in GSH content and decrease in LPO level due to radiation. A significant increase in the serum alkaline phosphatase activity and decrease in acid phosphatase activity was observed in AV leaf extract pretreated irradiated animals during the entire period of study [145].

Similar observations were also found by Kumar et al., in 2007, the antioxidative and genoprotective role of ethanolic extract of AV was observed on radiation induced damage by assessing cellular, biochemical and chromosomal parameters (e.g., GSH content, LPO level, acid and alkaline phosphatases activity and chromosomal aberrations) in Swiss albino mice. This study also reiterated the free-radical scavenging mechanisms of AV extract responsible for protection against radiation induced damages [146].

- **Safe doses for human being**

A clinical study was conducted with 24 volunteers, administered with 0.5–16 mg Vasicine by intravenous route with an aim determine the acute toxicity potential and safe dosage range in human. Vasicine was well tolerated at these doses and no undesirable effects were observed in the volunteers up to 16 mg dose level [146].
• **Abortifacient activity**

As mentioned above Vasicine from AV has been reported to be abortifacient too. Gupta et al. (1977) investigated the uterotonic activity on uterus of different species of animals in different hormonal states both *in vitro* and *in vivo* [54]. It was found that, in a comparative study with methergen and pitocin, the uterotonic effect of Vasicine was similar to that of these two known oxytocics. As, the changes in the responsiveness of uterus to Vasicine varied according to its hormonal status, similar changes in the responsiveness of uterus were observed with methergen and pitocin also. They suggested that, Vasicine might be acting through the release of prostaglandins. Also, it was suggested that, Vasicine being a respiratory stimulant can antagonize the respiratory effects of narcotic analgesics when used in labour and it is also useful to control post partum hemorrhage. Chandokhe et al. (1978) had also reported this compound to mediate the effect via prostaglandins [147]. Further Gupta et al. (1978) have reported Vasicine to enhance the uterotonic effect in rats and guinea pigs depending upon the stage of pregnancy and prior priming with oestradiol and supported the idea of prostaglandins being the mediators again [148]. But, in rats they reported that, it did not (5 - 15 mg/kg intra-peritoneal on 18th and 16th day of pregnancy) have abortifacient effect. In case of guinea pigs, when Vasicine (30 mg/kg) was given on late stage of pregnancy, 50% abortion was observed. When Vasicine is primed with oestradiol (10 mg/kg vasicine, 50 μg oestradiol), 3 out of 8 at early stage, 50% at middle stage and 10 out of 12 aborted. Vasicine, at a dose of 1 mg itself has been shown to have no effect on contractions [148]. It has stimulatory effect on F2α and PGE1 evoked contractions in isolated rat uterus. Vasicine increased the contractions when it was given before PGE1.

Further Gautam et al. (1982) studied the effect of Vasicine and (+) sotalol and deoxysotalol on oxytocin induced contractions [149]. They found that, (+) sotalol (10 μg/ml), deoxysotalol (10 μg/ml), and Vasicine (1 μg/ml) produced marked potentiation in the contractile responses of oxytocin, while (+) sotalol and deoxysotalol did not potentiate the responses of oxytocin on mammary strip (selective for uterus tissue), Vasicine HCL potentiate these responses. Further aqueous or 90% ethanolic extracts of leaf of *Adhatoda vasica* has been reported to be 100 % abortive on rats after 10th day of insemination, at a doses equivalent to
175 mg/kg of the starting dry material [150].

As, in case of bronchodilation, synthetic derivative of Vasicine: bromhexine, was also worked upon for its abortifacient activity in rats. Bromhexine modifies the onset, appearance and regulation of cascade of glycol/sialoproteins and this may interfere with the events leading to implantation of the trophoblast. Kinetics of bromhexine-mediated down regulation of focal adhesive molecules (glycol/sialoproteins) of uterus and trophectoderm affecting conception in the rat was studied by Singh and Malaviya (2006) [151]. 10mg/kg bromhexine was given to the ‘day 1’ pregnant rats twice daily for 3 days. Bromhexine inhibited the incorporation of sialic acid, galactose, glucose and glucosamine into proteins of pregnant rats, making protein more susceptible to proteolytic degradation. Bromhexine had shown interference in blastocyst attachment, conception, reduction in number of implantation sites and dwarfing of fetuses and hence, it was suggested to be a potential candidate for anti-implantation. As an additional advantage of using Vasicine during induction of child birth or during abortion is that, it helps to control post-partum hemorrhage. Atal et al. (1982) [152] experimented with animals and reported that repeated oral and intramuscular administration of Vasicine hydrochloride resulted in an increase in platelet count in normal rats, mice, rabbits and dogs. There was no effect seen on Hb amount, RBC and WBC number. The increase in platelet number was found to be associated with hyperplasia of megakaryocytes. They suggested that, Vasicine can be used for controlling the capillary hemorrhages and correction of drug induced bone marrow depression.

On the basis of the above discussion, probable mechanism and targets of action of AVE and Vasicine have been presented schematically for respiratory disorders in figure 2.7. As shown in the figure, in smokers the toxic components of tobacco smoke goes into the lung alveolar through lung that leads to an increase ROS and RNS level, that are produced: superoxides, hydrogen peroxide, hydroxyl radical and nitric oxide radicals, respectively. The level of these oxidants is controlled by antioxidants like SOD, and Catalase in the cells. But, in smokers, there occurs a tremendous rise in free radical formation and when the cellular antioxidants get depleted after continuously scavenging the oxidants, the cell faces a state of OS
(Figure 2.7). In order to handle such a situation, scientists have tried to analyze and successfully identified numerous medicinal plants with strong antioxidant property. As, *Adhatoda vasica* and its main phytoconstituent Vasicine have been reported to be an anti-oxidant and anti-inflammatory they may also show promising effects in respiratory ailments caused due to increase in oxidative stress. Therefore, the plant *Adhatoda vasica* and Vasicine were chosen for the present study to analyse their effects in tobacco smoke induced toxic condition and their possible mechanism in the present study.

![Diagramatic representation of probable mode of generation of OS in human lung alveolar epithelial and machrophage cells by tobacco smoke and its prevention by Adhatoda vasica and its active compound Vasicine.](image)