CHAPTER-II

REVIEW OF LITERATURE
REVIEW OF LITERATURE

2.1 Information about Cardiovascular disease

2.1.1 The Heart

The hollow muscular organ located behind the sternum and between lungs. Its rhythmic contractions move the blood through the body (KLM E Medical Dictionary, 2000). The term cardiac means “related to the Heart” and comes from the Greek ‘Kardia’ for ‘heart’. The heart is composed of cardiac muscle, an involuntary muscle tissue which is found only within this organ (The American Heritage Stedman’s Medical Dictionary, 2000). The average human heart has 72 beats per minute.

Structure:

In the human body, the heart is usually situated in the middle of thorax with the largest part of the heart slightly offset to the left, underneath the breastbone. The heart is usually felt to be on the left side because the left heart (left ventricle) is stronger (it pumps to all body parts). The heart is enclosed by a sac known as the pericardium, made of dense fibrous connective tissue, and a double membrane structure containing a serious fluid to reduce friction during heart contractions (the serous pericardium). The mediastinum, a subdivision of the thoracic cavity, is the name of the heart cavity. In normal adults, the mass of the heart is 250-350g (9-12oz) or about three quarters the size of a clenched fist. It consists of four chambers, two upper atria and the two lower ventricles (Ballard, 1997).

2.1.2 Functioning of human heart

The function of the right side of the heart is to collect de-oxygenated blood, in the right atrium, from the body and pump it, via the right ventricle, in the lungs (pulmonary circulation) so that carbon dioxide can be dropped off and oxygen picked up (gas exchange). The left side collects oxygenated blood from the lungs into the left
atrium. From the left atrium the blood moves to the left ventricle which pumps it out to the body. Starting in the right atrium, the blood flows through the tricuspid valve to the right ventricle. Here it is pumped out the pulmonary artery to the lungs. Then the blood flows back through the pulmonary vein to the left atrium. From there it goes to the aorta through semi-lunar valve. The aorta forks and the blood is divided between major arteries which supply the upper and lower body. The deoxygenated blood then travels to the venules which coalesce into veins; then to the inferior and superior venae cavae and finally back to the right atrium where the process began. The heart is effectively a syncytium, a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges (Avraham and Koop, 1989).

2.1.3 Cardiovascular Disease

Cardiovascular disease is also known as heart and circulatory disease and this term covers all diseases that affect the heart and circulatory system. There are about nine risk factors associated with heart attacks; Inactivity, Obesity, High blood pressure, High levels of cholesterol, Stress and Tension, Smoking, a family history of heart disease, Age (as an individual gets older, the risk increases) and Individual gender (British Heart Foundation, 2008). Cardiovascular fitness is often thought as the most important component of all physical fitness components. It includes the efficient operation of both the circulatory and respiratory systems. These two systems improve with exercise and decline with inactivity.

In severe cases of cardiovascular ailment a build-up cells, fat and cholesterol, often referred to as ‘plaque’, clogs the arteries, impending the free flow of blood. Overtime, the blood vessels become blocked, and a heart attack or stroke can occur.

The major cause of heart ailment is the combination of ‘Nature’ and ‘Nurture’ (genetic predisposition; nature and urbanization with offence; nurture):
• Genetic predisposition can be seen by increased levels of lipoproteins; with higher lipoproteins levels the harmful effects of environment are highly magnified.

• Urban life styles; people nowadays have become sedentary and have adopted western life style causing decreased physical activity. Increased consumption of calories and saturated fats, results into obesity, insulin resistance and antherogenic lipid abnormalities which have a synergistic effect with lipoprotein (Lp a). High Lp, high triglyceride, high levels of bad cholesterol and low levels of good cholesterol predispose to heart disease and heart attacks (Johansson, 1998).

• Junk Food, tobacco use, high blood pressure and liquor consumption in large quantities also are the major factors for this ailment.

• Recent evidence suggests that impaired foetal nutrition reflected in small birth size, results in programmed susceptibility to adult cardiovascular disease (Reddy and Yusuf, 1998). This is the reason why people among economically weaker sections are equally vulnerable to heart attacks.

• Hypertension, Diabetes Mellitus, Dyslipidaemia and family history of premature antherosclerosis are predictive of subsequent myocardial infarction and stroke in women as well as men although relative impact of individual risk factors differ (Yusuf et al., 2004).

2.1.4 Types of cardiovascular disease

2.1.4.1 Coronary artery disease (CAD)

It is a condition in which the heart muscle receives an inadequate amount of blood because of an interruption of its blood supply. Depending on the degree of interruption, symptoms can range from a mild chest pain to a full-scale heart attack. Generally the symptoms manifest themselves when there is about 75% narrowing of coronary artery
Fig-2.1- Heart showing Antheroclerosis

Fig-2.2- Heart showing coronary artery spasm

(Source, Du Bose et al., 2000)
Insufficient blood flow to the heart muscle from narrowing of coronary artery may cause chest pain.
Two of the principal causes of CAD are atherosclerosis and coronary artery spasm (Johansson, 1998).

2.1.4.2 Antherosclerosis

It is the medical term for hardening or thickening of the artery walls. It is a situation characterized by the narrowing of the arterial wall with large number of smooth muscle cells and deposits of cholesterol and other substances in the portion of the vessel wall closest to the lumen.

The mechanism that initiates this thickening is not clear, but it is known that cigarette smoking, high plasma cholesterol concentration, hypertension, diabetes and several other processes increase the incidence and severity of the antherosclerotic process. In a very destructive and painless process the symptoms of antherosclerosis affects the cardiovascular system in general. This is usually progressive after leading ultimately to complete occlusion, Fig 2.1 (Du Bose et al., 2000).

2.1.4.3 Coronary artery spasm

It is a condition in which the smooth muscle of a coronary artery undergoes a sudden contraction, resulting in vasoconstriction. Although the causes of coronary artery spasm are not well known, smoking, stress and alcoholism are said to be triggering agents (Fig 2.2).

2.1.4.4 Hypertension

It is high blood pressure, which occurs when the pressure in the arteries is consistently higher than normal, indicating that the heart is working harder to pump blood through the circulatory system. The blood pressure of a normal adult is 120/80. Severe high blood pressure is diastolic pressure of 116 or higher and systolic hypertension is systolic pressure greater than 160 in those whose diastolic pressure is less than 90. High blood pressure may not produce symptoms until a major organ is damaged. People
Fig-2.3: Antherosclerotic artery showing Peripheral Arterial Disease (PAD)

Fig-2.4: Heart showing muscle damage and a blocked artery

(Source: Du Bose et al., 2000)
Coronary artery (supplies blood and oxygen to heart muscle)

Healthy heart muscle

Blood clot blocks artery

Plaque buildup in artery

Blocked blood flow

Dead heart muscle

Coronary artery

Heart muscle
suffering persistently high blood pressure are at a risk of a heart attack or stroke and even kidney failure. Hypertension causes a variety of problems.

- One of the most affected organs is the heart due to increased arterial pressure. It develops an adaptive increase in muscle mass.
- Its pressure also enhances the development of arteriosclerosis and heart attacks.
- The severe strain imposed on the cerebral arteries that supply the brain causes its weakening ultimately leading to rupture and causing brain haemorrhage.
- The Kidney are another prime target of hypertension, it causes narrowing of the human of the arterials of kidney. To combat this, kidney secretes renin which further raises the blood pressure and aggravates the problems (Kearney _et al._, 2005).

2.1.4.5 Peripheral Arterial Disease (PAD)

Peripheral arterial disease (PAD) is a form of antherosclerosis involving the arteries in the arms, legs, neck, kidneys and lower abdomen. This can cause pain and difficulty in walking (fig 2.3).

2.1.4.6 Heart Attack

It occurs when the blood flow to part of the heart is blocked, usually by a blood clot or a piece of dislodged plaque. If the blood flow to the heart is either blocked or significantly reduced for more than a few minutes, the part of heart muscle begins to die (Fig 2.4).

2.1.4.7 Heart Failure

Heart failure occurs when the heart fails to pump blood efficiently, robbing the body of the necessary blood or oxygen. Common symptoms of heart failure include ‘oedema’ and ‘pulmonary congestion’. Odema is swelling in the feet, ankles or legs and pulmonary congestion is fluid build-up in the lungs.
Fig-2.5: Artery of heart showing stroke or Transient Ischaemic Attack (TIA)

(Source: Du Bose et al., 2000)
2.1.4.8 Stroke or Transient Ischaemic Attack (TIA)

It occurs when a blood vessel feeding the brains becomes clogged, or bursts. As a result, that part of the brain and the part of the body controlled by it ceases to function. Three main causes of stroke are uncontrolled high B.P., smoking and heart disease (Du Bose et al., 2000).

2.1.5. Common symptoms of cardiovascular disease

According to W.H.O. report -2009, often, there are no symptoms of this disease – A heart attack or stroke may be the first warning of cardiovascular ailment. Symptoms of heart attack include pain or discomfort in the centre of the chest lasting for more than a few minutes, pain radiating out to the shoulders, jaw, neck or arms. In addition the person may experience difficulty in breathing or shortness of breath, feeling sick or vomiting, feeling lightheaded or faint, breaking into a cold sweat; and becoming pale. Women are more likely to have shortness of breath, nausea, vomiting and back or jaw pain. The most common symptom of a stroke are sudden weakness of the face, arm or leg, most often on one side of the body, sudden blindness in one eye, difficulty in speaking or understanding speech, difficulty in walking, dizziness, loss of balance or coordination, severe headache with no known cause and fainting or unconsciousness (Parks, 2008).

2.1.6 Statistical Analysis of Cardiovascular Disease in the World

According to American Heart Association (2007) cardiovascular disease is by far the number one killer in the United States. About 62 million Americans have same form of cardiovascular disease. In the year 1999 about 960,000 Americans died of cardiovascular Disease (CVDs). So CVD accounted for about 40.1% of all deaths in U.S.A.
It is the number one cause of death and disability in the United States and most European Countries. By the time the heart problems are detected its cause i.e. Antherosclerosis is usually quite advanced (Vanhecke et al., 2006).

According to British Heart Foundation, (2008), it is the most common cause of death in the U.K. and includes Coronary Heart Disease (CHD), angina, heart attack and stroke. CHD is the most common form of cardiovascular Disease (CVDs) in the U.K; with approximately 275,000 heart attacks reported every year and an estimated 1.4 million people suffer from angina; strokes are the leading cause of disability in the U.K. 80% of global CVDs related death now occur in low and middle income nations which cover most countries in Asia (Joshi et al., 2006). In India, in the past 5 decades, rates of CHD among urban populations have risen from 4% to 11%. In urban china, this percentage rose by 53.4% from 1988 to 1998 (Enas et al., 2000). Australian Bureau of statistics (ABS), 2007 estimated that 5.7% of population (785,000 people) reported having a long term cardiovascular condition and it is Australians number one killer, which in 1992 accounted for 46,752 premature deaths.

World Health organization (W.H.O.) gave a report in 2009 that globally cardiovascular diseases are the number 1 cause of death and is projected to remain so. An estimated 17.5 million people died from cardiovascular disease in 2005, representing 30% of all global deaths. If current trends are allowed to continue, by 2015 an estimated 20 million people will die from cardiovascular diseases.

2.1.7 The present status of disease in India

In India as many as 30 million people have known to be heart patients in the year 1995. By the end of 2010, one million Indians were suspected to be suffering from some kind of heart disease. And by 2015, heart disease is likely to be the single largest cause of death in the country. India has the highest rate of mortality and morbidity from heart
Fig-2.6: Report of World Health Organization (W.H.O), 2009

on Human Mortalities caused by different diseases in

the World
Communicable diseases, maternal and perinatal conditions and nutritional deficiencies
Cancer + Chronic respiratory diseases + Diabetes
Cardiovascular diseases 30%
Other chronic diseases 9%
Injuries 9%

W.H.O
attacks in the world (World health Report, 2010). Every 6th patient with heart attack is between 40 years of age. Young Indians have a 5-10 fold higher rate of heart attacks and death as compared to urban population. The most common form of heart disease which affects children and young adults in our country is rheumatic fever and rheumatic heart disease.

Epidemiologists in India and international agencies, such as W.H.O., have been sounding an alarm on the rapidly rising burdens of CVDs for the past 15 years. It is the world’s largest killer, claiming 17.1 million lives every year. The reported prevalence of coronary heart disease (CHD) in adult surveys has raised 4-10 folds over the last 40 years, to a present level of around 10% even in rural areas the prevalence has doubled over the past 30 years, to a present level of around 4%.

People among economically weaker sections are equally vulnerable to heart attacks. Low nutrition and low birth weight among under nourished Indian women could be an important contributing factor for development of heart disease (Reddy, 2003).

A comparison of per-capita income profile and death rates in Punjab, Maharashtra, Andhra Pradesh and Orissa in 1998 reveals that as per capita income profile progressively ‘decline’ from Punjab to Orissa, the probability of death between the ages of 15 and 60 years progressively ‘Rises’ (19%, 27%, 34% and 38% respectively), (World Bank, 2003).

India accounted for 17% of all CVD deaths in the world. CVDs in India alone accounted for around 2.4 million deaths, in contrast to nearly 3.2 million deaths in all the industrialized countries together, (Murray and Lopez 1996), (Table-2.1and 2.2).

Asian Indians are at highest risk of heart disease in the world (Josh et al., 2007). As lifestyle changes in India, heart disease is hitting epidemic proportions. Now India wakes up to the threat Cardiovascular Diseases, which is seen by 2007 Union budget
### Table 2.1: Contribution of NCDs and Injuries to Mortality in India (1990-2020)

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Cause-specific proportionate Mortality (as % of total mortality)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1990</td>
</tr>
<tr>
<td>Total NCD</td>
<td>..40.4..</td>
</tr>
<tr>
<td>Cardio Vascular</td>
<td>..24.2..</td>
</tr>
<tr>
<td>Diseases</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2.2: Middle Mortality due to NCDs in India (1990–2020)

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Percentage of cause specific mortality occurring in the age groups of 30-69yrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1990</td>
</tr>
<tr>
<td>Total NCD</td>
<td>..48.9..</td>
</tr>
<tr>
<td>Cardio Vascular</td>
<td>..48.2..</td>
</tr>
<tr>
<td>Diseases</td>
<td></td>
</tr>
</tbody>
</table>
presented by the Finance Minister of India, who allocated 4 million U.S. dollars to a National programme for the prevention and control of cardiovascular diseases, strokes and Diabetes. It is the first ever attempt at initiation a national programme to counter the hitherto neglect to the threat of CVDs.

2.2 Preference for Selection of Plant Species

All the selected plant species are found to be quite useful in combating problems regarding ailing heart, hypertension and blood vascular system.

*Withania somnifera*

This is a unique herb having antistress and adaptogenic action that leads to better physical fitness and helps cope with life’s daily stress (Singh *et al.*, 1982). Hence it can be useful in treating hypertension. It is a strong sedative and tonic and has a rejuvenating effect on the body (Chopra *et al.*, 1986). It has biochemically heterogeneous alkaloids, which has shown great value when studied against bloodvascular muscles (Malhotra *et al.*, 1961; Mishra, 2001). *Withania somnifera* is reported to provide cardioprotection. It has anxiolytic (anxiety reducing) and antidepressant actions (Mohanty *et al.*, 2008).

There are few preliminary studies available on the effects of *Withania sp* on hemopoetic system and cardiopulmonary system to form a basis for further studies but not enough evidence to provide a firm scientific basis for definitive therapeutic uses.

*Ocimum sanctum*

Holy basil is a sacred plant throughout India but has shown great therapeutic potential. It has cardiotonic, blood purifier properties and can be useful in the treatment of depression and exhaustion (Chopra *et al.*, 1986). It shows great antioxidant property (Banerjee *et al.*, 1996). Fixed oil of this plant is comparable to aspirin given to prevent blood clotting (Singh *et al.*, 2001). *Ocimum sanctum* has shown anti- stressor effect by lowering cholesterol level in animal studies (Sen *et al.*, 1992). As a cardiac depressant
Plate-1

Fig a: *Ocimum sanctum* plant bearing flowers.

Fig b: A colony of *Ocimum sanctum* plants (Kali Tulsi).

Fig c: A mature tree of *Emblica officinalis*.

Fig d: *Emblica officinalis* tree bearing fruits.

Fig e: A colony of *Boerhaavia diffusa*.

Fig f: *Boerhaavia diffusa* plant bearing pink flowers.

Fig g: *Boerhaavia diffusa* plant bearing white flowers and fruits.

Fig h: Roots of *Boerhaavia diffusa* plant.

Fig i: A small plant of *Withania somnifera*.

Fig j: *Withania somnifera* plant bearing flowers.

Fig k: A colony of *Withania somnifera*.

Fig l: Full grown *Withania somnifera* plant.
(Sharma et al., 2002), having a positive effect over blood pressure (Bharghava and Singh, 1981) and also as a detoxicant (Panda and Kar, 1998). Tulsi can be exploited in curing heart ailments.

**Boerhaavia diffusa**

The root of this plant has shown great therapeutic uses especially in relation to heart problems. It is a great antistress agent (Mishra, 1980; Chandan et al., 1991; Rawat et al., 1997). The alkaloid fraction of the root has evidenced a dramatic effect in reducing elevated levels of cortisol under stressful conditions. It lowers blood pressure (Gaitonde et al., 1974). It cleanses blood, as cardiotonic it tones, balances, strengthens the heart (Singh and Udapa, 1972). *Boerhaavia* extract has significant antioxidant activity (Mungantiwam, 2000; Satheesh, 2004; Taylor, 2005). It is a Cardiac depressant (Thakur et al., 1989). All these properties of *Boerhaavia* suggest it can be helpful in curing cardiovascular disease.

**Emblica officinalis**

Amla provides a broad-based approach in preventing cardiovascular damage through its powerful cholesterol regulating and multifaceted anti-oxidant properties (Jacob et al., 1988). *Emblica* has the ability to lower cholesterol and actually reduces plaque in clogged arteries caused by high cholesterol levels in some animal studies (Thakur 1985). Amla is a potent scavenger of free radicals (Chaudhari and Ratan, 2004). The use of amla as an antioxidant has been examined by many scientists (Bhattacharya, 1992; Nadkarni et al., 1954). It is also useful as a blood purifier (Scartezzini et al., 2006).

### 2.2.1 Information about the selected plants

**Ocimum sanctum** Linn

vishnupriya. Sanskrit: Tulasi, surasa, Brinda, Parnasa. Habit- It is an erect herbaceous, much branched, soft hairy annual. Habitat- Cultivated throughout India.

Chemical composition: Eugenol, nerol, caryophyllene, terpeinen-4-ol, decylydehyde, y-selinene, B-pinene, camphene and a-pinene from essential oil. Plant contains citric, tartaric and malic acids.

Parts used: Leaves, seeds and roots.

Ayurvedic properties:

*Tulasi katukatikata Hirdyoshadna Dahpittajit, Dipani Kushthhrstshwaspashravarukk kaphvatjit.* (BhavPrakash Nighantu).

Actions: Vatahara, Kaphaghna, Sitahara, Jvaraghna, Dipana, Uttejaka, Hrdya Parsvasulahava, Kushthara.

Therapeutics: Tulsi is aromatic, carminative, antipyretic, diaphoretic and expectorant. It has been found to be very effective in treatment of viral encephalitis and tropical pulmonary eosinophilia in children. Plant is used in snake bite and scorpion sting. Fresh roots, stems and leaves are bruised and applied to the bites of mosquitoes. Decoction of roots is used as diaphoretic in malarial fevers. Leaves are expectorant. Juice of leaves is diaphoretic, antiperiodic and used in catarrh and bronchitis. Infusion of leaves is given in malaria, used as stomachic, in gastric disorders of children and in hepatic affections. It improves appetite, afflictions of ear, destroys intestinal worms and cure skin disorders. Dried leaves, powdered and used as snuff in ozaena. Seeds are given in disorders of genito- urinary system (Joshi, 2006).

*Boerhaavia diffusa* Linn

Visakha, Kathilla. Habit- It is a very variable, diffusely branched, pubescent or glabrous, prostrate herb. Habitat - a weed found throughout India.

Chemical Composition: Hentriacontane, B-Sitosterol, ursolic acid, punarnavine-land 2, myricyl alcohol, myristic acid, oxalic acid and ackaloids. Its polysaccharide consists of glucose, xylose, glucuronic acid, galctose, L-arabinose and L-rhamnose and a glycoprotein.

Parts used: Whose herb and root.


Punernavavruna Tikta Katupaka himalaghu,
Vatala Grahini shlempitarakta Vinashini. (Dravyaguna)

Katukashayanurasa Pandughani Dipani Sir,
Shopanilgarshleshmehri Varnayodarpranut. (BhavPrakash Nighantu).


Therapeutics: Punarnava is pungent, bitter, astringent, hot and laxative. It is stomachic, diuretic, diaphoretic, expectorant, antipyretic and cardiotonic. It stimulates function of heart and kidney and is a specific for jaundice, diabetes and general debility. It is a rejuvenative drug. It is used in epilepsy, pain in abdomen due to congestion of blood and dysentery. The whole plant, fresh or dried, is the source of drug punarnava. Root is diuretic, laxative, anthelmintic and febrifuge. It is used as expectorant in asthma, anaemia, jaundice, scanty urine and internal inflammation. Root is useful for restoration of virility in man. Its poultice mixed with palm oil is applied to boils. It is an antidote to snake venom. Leaf ash and roots are taken to cure night blindness. Alcoholic extract of roots and plant is spasmylytic, leaves are used in ophthalmia and in eye wounds, in
muscular pain, in dropsy and gonorrhea, to purify blood and hasten delivery. Dry and powdered leaves mixed with brassica oil used for external application on itch and eczema. Flower and seeds are contraceptives in Ayurveda. It is effective in cases of oedema and ascites resulting from early cirrhosis of the liver and chronic peritonitis. It is useful in abdominal tumors and cancer (Joshi, 2006).

**Withania somnifera**


Chemical Composition: Roots contain several pyrazole alkaloids. Withasominine, steroidal lactones, withaferin A and withanolides. They also contain starch, reducing sugars, hentriacontane, glycosides, dulcitol, and withaniol. Parts used: Roots, leaves, fruits and seeds.


*Ashwagandha Katushna syatikta Ch. Madhgandhika,*

*Balya Vathara Hanti Kasshwasakshayvranan.*

*Ashwgandhanilshleyshmashvithr Shothakshayyapha,*

*Balya Rasayni Tikta Kashayoshna-atishukrlaa.* (BhavPrakash Nighantu).

Actions: Vataghna, Kaphaghna, Sothahara, Brihana, Balya.

Therapeutics: Asvagandha plant is sedative, stimulant, aphrodisiac and helps toning up of uterus of women. Internally it is given in marasmus in children. Externally it is used in the treatment of inflammatory conditions, ulcers and scabies. Root is adaptogenic, diuretic and tonic. It is useful in cough, dropsy, hiccups, leucorrhoea and menstrual troubles. It restores loss of memory and is used in cases of nervous exhaustion,
spermatorrhoea and senile debility. Powder of root mixed with equal parts of ghee and honey is beneficial in impotency or seminal debility. Decoction, boiled with milk and ghee promotes nutrition. Leaves are bitter, antipyretic and anthelmintic. Bruised leaves and ground root is locally applied in carbuncles, painful swellings and ulcers. Fruit is sweet, applied to wounds and is used in asthma, biliousness and strangury. Ripe fruits are sedative. Round capsular fruit is used in fresh state as an emetic and when dried is used as a stomachic. In small doses it is a remedy in dyspepsia and flatulent colic. Seeds are, useful in lumbago and opthalmia. They lessen inflammation of piles (Joshi, 2006).

**Emblica officinalis** Gaertn


Chemical composition: Fruits and leaves contain tannins, polyphenolic compound, 1,3,6-trigalloylglucose, terchebin, corilagin, ellagic and phyllenbic acids also alkaloids phyllantidine and phyllantine,. Leaves and stem yield lupol and beta- sitosterol. Seeds contain linoleic acid, ellagic acid and lupeol from roots. Fruits are rich source of vitamin C. Parts used - dried fruit, seed, leaves, root, bark and flowers.

Ayurvedic properties: Rasa- Amla, Madhura, Katu, kasaya.


*Amlakam Kashayamlam Madhuram Shishiram Laghu,*

*Dahpittavahimeh shophghnam ch Rasayanam.*

*Katunadhurakashayam Kinchidamlam Kaphghnam,*

*Ruchikar matishitam Hanti Pittastratapam.*

28
Shramvanmanvivandhadhaman Vishtibhadosh,

Prashamanamritabham Chamlakya Phalam syath. (BhavPrakash Nighantu).

Action: Vatahara, Pittasamaka, Tridosahara.

Therapeutics: Various plant parts of Amla are used in toothache, sores, fever, epilepsy, pimplers, tubercular fistula, gonorrhea and convulsions. Root and bark are astringent. Fresh roots are used as a remedy for jaundice. Leaves are cerebral and gastro-intestinal tonic, cardiotonic, aphrodisiac, antipyretic and antidiabetic. Leaf extract is antibacterial. Decoction of leaves is useful for ulcers in mouth. Flowers are cooling, refrigerant and aperient. Fruit is acrid, diuretic and laxative; it is a pronounced expectorant and has anticancerous properties. It is especially good for abundant growth of hair. It has been found to be effective in the treatment of peptic ulcer and scurvy. Fruit juice with lemon juice and sugar is taken for arresting bacillary dysentery. In combination with iron it is used for anaemia, jaundice and dyspepsia. Exudation from incisions on the fruit used as external application for inflammation of eye. It is a constituent of many ayurvedic combinations such as Triphala used for constipation. Arogyawardhani used in viral hepatitis, chawanaprash used as a general tonic (Joshi, 2006). Anticholesterolaemic and antiantherogenic effects of the fruits have been studied by Thakur and Mandal (1984).

2.3 References from other sources for cure of the disease

2.3.1 Remedies for the Disease

This includes prevention and remedies through various mediums as allopathic drugs, yoga, ayurveda, supplements and other mediums.

2.3.1.1 Prevention

Attempt to prevent cardiovascular disease takes the form of modifying risk factors. Some, such as family history or age cannot be modified; but there are other risk factors which are strongly associated with cardiovascular diseases but are potentially
reversible or can be modified. These are high blood cholesterol levels; especially saturated and trans-fats (LDLs), cigarette smoking, low levels of HDLs, high triglycerides, being overweight, tobacco use, diabetes, high blood pressure, inactivity, high alcohol consumption and high level of anxiety or stress (British Heart foundation, 2008).

As said, 'An ounce of prevention is better than a pound of cure.' There are three types of prevention; primary prevention, in which one comes in the danger zone of cardiovascular disease by having family history of heart disease or due to old age and being overweight, but there is no near and real threat.

Secondary prevention, in which a person may be having high Blood Pressure or diabetes, anxiety or undergoes substance abuse as tobacco, alcohol or cigarette smoking, is obese and has a bad life style leading to stress in daily life and hence there is a real threat of CVD.

Tertiary prevention, in which the person is suffering from some form of heart disease like having a weak heart, irregular heartbeat, or chest pain or hypertension, here a long life and future problems can be solved by undergoing some preventive measures.

In all three types of prevention one has to make small changes in one's lifestyle and combat the threat shown by the number one killer disease of the world (British Human Nutrition Research, 2008).

Dietary changes include:

- **Fat reduction:** All cholesterol is not bad. There are two types of cholesterol in the blood stream. LDLs and HDLs. LDLs create the build up in the arteries while high HDL levels are a good sign showing the heart is not at risk of CVDs. So reducing saturated fat can help to reduce blood cholesterol levels. Trans-fatty acids are naturally occurring in meat, dairy products, margarines hydrogenated vegetable oil. We can modify fat
intake by; reducing butter and other spreads. Choosing lean cuts of meat or trimming
the fat off. Grill, bake or steam instead of frying. In place of using butter, using
unsaturated oils as sunflower, rapeseed or olive oil would be better in lowering lipid
levels. Reducing processed food, which has high level of trans-fats. Eating fish like
sardines, tuna, salmon, which have HDLs.

- Essential fatty acids: Such as omega-3 S, which are found in oily fish, they have been
  shown to reduce the risk of CVD, by lowering blood triglycerides.

- Stanols and sterols: Certain plant derived compounds called stanol or sterol esters
  have been shown to reduce cholesterol levels; like soya dairy alternatives, yoghurts are
  now available containing these products.

- Fruits and vegetables: Fruits and green vegetables which are rich in many essential
  nutrients as vitamin C and E and many micronutrients i.e. Magnesium, Zinc, Iron,
  Potassium etc, should be consumed. They work as antioxidants and help in heart
disease.

- Whole grain and Fibre: U.S. researchers have shown that diet rich in whole grain
  food can reduce the risk of CVD by up to 30% (American Heart Association, 2007).
  Soluble dietary fiber, found in oats, beans and pulses can help to lower LDL cholesterol.
  Soya protein has been associated with reduction in LDL cholesterol and it is also an
  excellent substitute for meat (Vanhecke et al., 2006).

  Tobacco control is very essential for reducing the risk of cardiovascular ailments.
  It may be reduced by taxation, regulation and education. Blood glucose level should be
  maintained, in case of diabetes. One should consume moderate amount of alcohols. High
  blood pressure should also be managed, by reducing the salt. Meditation can be practiced
to relax mind and avoiding stress and hypertension. Physical activity should be increased.
Weight reduction is also important; a combination of healthy diet and exercise is a method to keep a check on body weight (Rastogi and Vaz, 2004).

2.3.1.2 Allopathic Drugs

There are a range of drugs to cure heart ailments depending on the type of heart ailment and its severity.

- Cholesterol lowering drugs e.g.- statins and fibrates, these medications have additional benefits aside from lipoprotein profile improvement.
- Tranquilizers and Anti-depressant-in case of hypertension. e.g.- various sedatives; Diazepam group of drug; valium.
- Aspirin- thinning of blood, as it has shown to decrease the clot formation, which can lead to myocardial infarctions and strokes, it is routinely prescribed for patients with one or more cardiovascular problems. (Vanhecke et al., 2006)
- Diuretics- With addition of beta blockers, if required.
- Estrogen- for coronary heart patients.
- Surgery- is also performed in severe cases for saving life- like angiography, angioplasty, stenting, by-pass surgery, ballooning of arteries, dye treatment for blockage etc., (Reddy, 1993).

2.2.1.3 Remedies through Yoga

This age old art has shown great usefulness in maintaining a healthy heart. There are different “Asans” and “Kriyas” for different types of ailments for heart.

(i). Coronary artery spasm

- Asanas – Pawan Muktasana and Vajrasana.
- Pranayam – Nadishodhan and Ujjayai pranayam.
- Kayatsarga – 40 minutes, twice a day.
(ii). Hypertension

- Asanas – Tadasana, Shashankasana
- Pranayam – Chandrabhedi pranayam
- Shatkriyas – Jalneti and Shankh-prakshhalan.

(iii). In General, for weak heart

- Anulom – Vilom and Bhramari
- Anupreksha – Contemplation of fearlessness.
- Shava asana – For mental piece, lowering down stress and anxiety
- Shitali Pranayam – for lowering of Blood pressure

Yoga can be very useful in treating illness due to psychosomatic factors and cardiovascular ailment is one of them. An ailment in which strain and stress play a major role (The word psychosomatic bring comprised of two Greek words, ‘Psyche’ and ‘soma’ which means mind and body). The psychosomatic factors stress and strain can be greatly reduced by regular and disciplined yogic treatment (Sinha.1970; Swami Ramdev, 2004).

2.3.1.4 Remedies through Ayurveda

The 2000 year old Indian system of medicine has shown miracle for treatment of cardiovascular disease. Medical doctors have discussed the efficacy of Ayurvedic drugs in relation to failing heart. *Terminalia arjuna* and *Jharmohara* have shown alternative for by-pass surgery. In 66% of the cases the levels of chest pains (angina) and fatigue came down dramatically (Dwivedi and Agarwal, 1994). Plants have contributed immensely in the development of certain allopathic cardiac drugs e.g. Dioxin – *Digitalis purpurea*, *D. lanata*, *Strophanthus gratus*, *Thevetia nerilifolia*, Codeine- *Papaver somniferum* and Aspirin – *Fillipendula ulmaria*.

Plants are also the major component of Ayurvedic medicines. Some ayurvedic medicines which are prescribed in heart ailments are:
(i) Divya Mukta Vati- Lowers down high blood pressure and anxiety.

(ii) Divya Hardayamrta, Divya Arjuna Kvatha- It strengthens the heart, removes the blockage of the arteries and also relives the frequent occurrence of angina pain (Swami Ramdev, 2004).

2.3.1.5 Remedies through other medium

2.3.1.5.1 Homeopathy

It is an important alternative medium in treating ailments. It is much practiced in India due to its efficacy. There are various homeopathic medicines which are given in case of cardiovascular ailments:

(i) Chest Pain (Angina Pectoris): Arnica 200, 1000; is given till the pain subsides. Lithium 30 and Spongia 200 in severe cases of angina.

(ii) Antherosclerosis:

- Crataegus mother tincture - is given when small physical effort causes great discomfort.
- Glonoine 200 - when both heart and brain receives throbbing pain due to less supply of blood.
- Opium 200- mind could not work properly.

(iii) Hypertension / High Blood Pressure:

- Aurum metallicum 30, 200 – for irregular heart beat.
- Glonoine 200 – A person cannot take up stairs and feels great fatigue.
- Lachesis 200 – Chronic hypertension, breathing trouble.

(iv) Aortic valve stenosis:

- Arsenic iodatum 30 – for vasodilation.
There are several medicines in homeopathy which are symptoms based and accordingly given by the homeopath Doctor. Few are stated above (Boericke, 2002; Clarke, 2000).

2.3.1.5.2 Supplements

- **Coenzyme Q10**: it is an excellent supplement for the heart. This chemical is a substance normally found in the body, but coenzyme Q10 is often deficient in people with irregular heartbeats, while supporting the functions of the heart, it also bolsters cardiac muscles strength and improves oxygen supply to the heart.

- **L-Carnitine**: in order to function properly and fully the muscles in the heart require adequate amounts of the substance carnitine.: L-Carnitine builds stamina while lowering the levels of triglycerides, LDL and cholesterol. It raises HDL level in the body. It is synthesized in the body from amino acids lysine and methionine aided by vitamins B3, B6 and C in the process.

- **Vitamin C**: World’s greatest chemists named Linus Pauling advocated the use of high doses of vitamin C to protect heart from arteriosclerosis as he was sure of its efficacy. Plaque formation is due to over stress when there is lack of Vitamin C. LDL (the Molecule Lp(a)) increases the risk of heart attack and stroke by 70% and vitamin-C lowers LDL (Fonorow, 2004).

- **Magnesium**: Many heart patients have very low levels of the essential mineral magnesium in their blood, hence supplementation using this mineral should be considered for heart ailments. The spasms in the coronary artery resulting in angina attacks are often due to magnesium deficiency.

- **Vitamin E**: It is often recommended as a supplement for heart disease as it improves muscular function and strengthens arterial walls. It improves oxygen supply to the
cells. Its antioxidant action prevents free radicals from damaging cells similar to vitamin C (Vanchecke et al., 2006).

2.3.2 Other Herbs for Cure of Heart Disease

From ancient times herbs has been used in the form of home remedies to cure or prevent heart related problems. Dietary consumption of these plants not only provides nutrients but also helps in cardiovascular Disease (CVD). Different parts of the world have suggested their ethnic uses; like Chinese herb, European herb and Russian herb in the form of tea, salad, mixed vegetable- dishes, in soup or taken as a whole fruit or vegetable; to help prevent and assist in curing CVDs. The medicinal effect of some well known herb are empirically proven and some of these plants are clinically studied as well (Kiss, 2001). Some of the herbs are mentioned below:

• *Adonis vernalis*: Ranunculaceae. Vernacular name – False Hellebore; Ox-eye; Red Morocca. It is a Russian folk medicine. Found in Russia, Bulgaria and Hungary. It is used as a remedy for irregular heart beat and weak heart. Homeopathic uses for *Adonis* include heart conditions.

• *Allium cepa* L.: Liliaceae. Vernacular name – Onion, piyaz. Since ancient times it is used in India in culinary preparations and in salad. The bulbs are acrid, sweet, aromatic, thermogenic, antibacterial and aphrodisiac. It is useful in normalizing the percentage of blood cholesterol by oxidizing excess cholesterol. It also lowers blood pressure (Kumar and Prajapati 2005).

• *Allium sativum* L: Liliaceae. Vernacular name,-Garlic, lashuna. It has thousands of years of historical medicinal usage; but it has also been the subject of numerous research studies, especially in regard to cardiovascular effects of garlic. It can increase the elimination of fats and cholesterol through the bile and overall cholesterol levels can be reduced. Garlic reduces the tendency of blood to form life threatening clots. It
exhibits moderate blood pressure-lowering effects also. Earliest Chinese and Egyptian dynasties also used it (Kumar and Prajapati, 2005).

- **Ammmi visnaga**: Apiaceae. Vernacular name - 'Khella'. It is a remedy for high Blood pressure, weak heart and irregular heartbeat. It is also used for angina and it combats spasms in the smooth muscles that line the walls of blood vessel. It improves circulation in the heart muscle and gives a mild boost to the heart’s pumping action. It is found in United States, Mexico and Chile.

- **Asparagus officinalis**: Asparagaceae. Vernacular name- Satavar. It is a European herb. It is an excellent food for strengthening the heart. It is a good medicine for a weak or enlarged heart. Steam cooking of this vegetable is quite beneficial.

- **Astragalus membranaceous**: Fabaceae, Vernacular name- Locoweed. It is a genus of plant that includes about 2,000 species of herbs and small shrubs. It is a Chinese herb, its root is used in teas, extracts or capsules. Milk-vetch and locoweed are examples of Astragalus. It is an adaptogen, which help protect the body against various stresses. It has been shown to lower blood pressure, a beneficial antioxidant during treatment of heart disease (Falch, 2009).

- **Atropa belladonna**: Solanaceae. Vernacular name- Black cherry, Deadly Night Shade. It is a remedy for irregular heartbeat and weak heart. Atropa acts primarily on the heart muscle and the smooth muscle in the digestive tract; relaxing the muscles and relieving spasms. A. belladona gained its name during the middle ages, when women used it to dilate their pupils. It is a common homeopathic medicine.

- **Berberis vulgaris**: Berberidaceae.Vernacular name-Holythorn; Barberry. Barberry has proved effective in reducing and normalizing heart rate. It is also used in lowering blood pressure. It is a European herb.
• **Capsicum annuum**: Solanaceae. Vernacular name—Cayenne. It contains capsaicin. Studies indicate it lowers cholesterol and lessens blood clotting while improving capillary strength throughout the blood stream. It helps in lowering blood pressure.

• **Carthamus tinctorius**: Asteraceae. Vernacular name—Safflower. Its oil has proved beneficial in lowering blood cholesterol. An emulsion with the trade name saffloxincipla is used routinely during myocardial infarction, cardiac ischaemia and hypertension.

• **Cassia absus** L: Caesalpiniaceae. Vernacular name—Bankultthi, Chaksu. It is helpful in conditions of ‘Kaphha’ and ‘Pita’. It is a wonderful remedy for bringing down high blood pressure (Brain, 2008).

• **Centella asiatica** L: Apiaceae. Vernacular name—Brahmi, Indian pennywort, Mandukkaparni. It is known as braintonic. It calms the heart and helps guard against heart attack. It is also useful in combating stress (Kumar and Prajapati, 2005).

• **Chrysanthemum coronarium, C. morifolium**: Asteraceae. It is native to Asia and Europe. It is used to treat circulatory disorders including atherosclerosis, in treatment for high blood pressure (Brain, 2009).

• **Commiphora mukul**: Burseraceae. Vernacular name—Guggul. It is taken to lower cholesterol levels and prevent hardening of the arteries. Chemicals in the resin is called guggulsterones, are responsible for its favourable effect on cholesterol and triglycerides. They not only lower levels of LDL, the bad cholesterol, but raise levels of the good cholesterol HDL. They also appear to reduce the stickiness of platelets in the blood, thus lowering the risk of dangerous clots.

• **Convallaria majalis**: Liliaceae. Venacular name—Lily of the valley, May Bells, May Lily. It is a remedy for circulatory disorders, heart palpitations, irregular heartbeat, and weak heart. It also lowers blood pressure and improves the heart’s efficiency.
• **Convolvulus arvensis** L: Convolvulaceae. Vernacular name—Beri, haranpadi, Bhadrabala. Roots of this plant are cathartic and seeds contain a fixed oil which is cardioprotective.

• **Crataegus oxyacantha** (Hawthorn): Rosaceae. Vernacular name—Hawthorn, white thorn. The best known herb for the heart in western herbalism is hawthorn; it is small tree or shrub that grows throughout the northern hemisphere. In Europe both homeopathic and allopathic doctors used this herb for various heart and cardiovascular ailments from the late 19th centuries (Hobbs, 1998). Clinical studies show, the leaves, fruit and flowers of hawthorn contain substances which act as antioxidants and which can help with symptoms of atherosclerosis. It contains wide range of flavonoids (vitexin), which helps in dilating coronary blood vessel. Hawthorn also prevents the build-up of cholesterol in the liver by encouraging the breakdown of cholesterol into bile acids. It affects intracellular calcium levels in the heart muscle, resulting in improved cardiac function and lowered spastic cardiac conditions (Kiss, 2001). Hawthorn is a common home-remedy for angina and weak heart in Europe. It is another circulatory superstar for CVDs.

• **Digitalis purpurea**: Scrophulariaceae. Vernacular name—Common foxglove, purple foxglove. Its effect on heart has been known from ancient times. It has a profound tonic effect on diseased heart. Foxglove's cardiac glycosides enable the heart to beat more strongly, slowly and regularly without requiring more oxygen. It is a remedy for weak heart and a source of Digoxin an active cardiac glycoside.

• **Ginkgo biloba**: Ginkgoaceae. It is a Chinese plant. It helps in circulation by dilating the blood vessel and is believed to protect the arterial walls. It also acts as a blood thinner (Falch, 2009).
• *Ilex paraguariensis*: Aquifoliaceae, Vernacular name-Mate, Paraguay Tea, Jesuit’s Tea. A remedy for heart palpitations, irregular heart beat and weak heart. It is a South American herb. Used as a tea preparation. It increases the force of heart contractions. It affects hearts rhythm and breakdown fats and sugars in the body.

• *Medicago sativa*: Labiatae, Vernacular name – Alfa alfa. It is helpful in trouble related with arteries, weak heart.

• *Polygonum multiflorum*: Polygonaceae. Vernacular name – Fo-Ti, He-shou-wu. It is a Chinese herb. A remedy for hardening of the arteries; High cholesterol. In traditional Chinese medicine, it is used to treat angina (Chest pain). The unprocessed root called FO-Ti is used in arteriosclerosis.

• *Pueraria lobata*: Fabaceae. Vernacular name-Kudzu, Ge-gen. It is a remedy for hardening of the arteries and high blood pressure. It is a Chinese herb. It contains ingredients to improve circulation in the muscles of the heart.

• *Rauwolfia serpentina*: Apocynaceae. Vernacular name- Sarpgandha. It is well known herb having great medicinal values. Root is useful in treating hypertension and anxiety. It is also useful in reducing high blood pressure (Vakil, 2005).

• *Salix acutifolia, S. alba, S. babylonica*: Salicaceae, Vernacular name-Willow. The bark contains ‘Salicin’, the herbal precursor of aspirin. It acts as a blood thinner to prevent blood clot. It has calcium channel blocker compounds (like verapamil, isoptin) compounds, that can prevent heart attack (Avraham and Koop, 1989).

• *Salvia miltiorrhiza*: Lamiaceae. Vernacular name-Salvia, ‘Danshen’. It contains several unique phytochemicals-(tanshinoates and salvianolic acids) with cardioprotective and antioxidant properties. It is a known Chinese medicine (Sugiyama, 2002).
• **Terminalia arjuna**: Combretaceae, Vernacular name – Arjuna. Since ancient times the bark is known for its great medicinal properties. It is a cardiotonic; reduces hypertension and act as a beta blocker (Bharani *et al.*, 1995).

• **Vaccinium myrtillus, V. scoparium**: Ericaceae, Vernacular name Bilberry. It contains flavonoides which is one of the most powerful antioxidants available. Fruit has about 0.8% anthocyanosides which has a toxic effect on blood vessels; strengthening the capillaries and making it a potentially valuable treatment for varicose veins and capillary fragility.

• **Valeriana officinalis**: Valerianaceae. Vernacular name- Valerian. It’s a European herb used for centuries to ease hypertension. It lowers anxiety and useful in panic attack.

• **Vitis vinifera**: Vitaceae. Vernacular name-Grape, Angoor. It is a very helpful fruit due to presence of vitamin C. There are certain phenolic compounds, which are found in grape skin; it protects the body from LDL cholesterol and thus helpful in antherosclerosis (Kiss, 2001).

• **Zingiber officinale Rose**: Zingiberaceae.Vernacular name -Ginger, Adarak. The raw ginger is acrid, thermogenic and laxative. It acts as a general tonic for heart (Kumar and Prajapati, 2005).

2.4 Availability and Distribution of selected plants

*Ocimum sanctum*

Availability of *O. sanctum* is throughout India, from Himalayas, ascending up to 1800 Meters to the Andaman and Nicobar islands. It has a wide range in the world also, found in tropical Asia, Africa, China, Europe, Malaya, Mexico, Panama, Philippines, Salvador, Sudan, Turkey, Iraq (Holtom and Hylton, 1979; Bown, 1995).
Possible locations are cultivated beds. It grows as a perennial plant in the tropics, but it is frost-tender and hence grows as a half-hardy annual in temperate zones (Chopra et al., 1986).

**Boerhaavia diffusa**

It is a weed found throughout India. Its distribution range is from tropical regions to warm-temperate areas worldwide (Wahi and Agarwal, 1997). This herb has about 30 genera and 300 species in tropics and subtropics. In Tropical America it has 6 genera and 13 species in China. Its range is in Africa, Australia, Brazil, Ghana, Haiti, Hawaii, Asia, Sudan, Dominican Republic and several Countries of the Middle East. Out of the 40 species common in Asia, 6 species are found in India; *B. diffusa*, *B Chinensis*, *B erecta*, *B repens*, *B rependa* and *B rubicund*. *Boerhaavia diffusa* is indigenous to India. It is found throughout the warmer parts of the country up to an altitude of 2000 M in the Himalayan regions. It is pioneer of bare areas, usually on dry sandy soils (Chopra et al., 1969). It grows well on wastelands and in fields after the rainy season. The plant is also cultivated to some extent in West Bengal (Heywood, 1978). The plant has perennial habit, which remains dry and dormant during summer and regenerates from the same old root stock in the rainy season (Chopra et al., 1923).

Possible locations are near disturbed areas, waste places, roadsides, dry pinelands, among scrub on tropical reefs (Flora of North America, 1987).

**Withania somnifera**

It is an erect, evergreen, perennial shrub. It grows in open and undisturbed lands in Eastern Asia, Australia and Africa. Its range is in Punjab, Sutluj valley, Sind Area and Baluchistan (Bown, 1995). It is also found throughout the drier parts of India in waste places and on bunds in Deccan, Mysore, Coimbatore, and Eastern Karnataka (Gamble,
1918). It is frost tender and is not hardy in temperate climates, but can be grown as an annual.

Possible locations are cultivated beds, sunny Edges, Dappled shade (Emboden 1979; Chopra et al., 1986).

**Emblica officinalis**

The emblic tree is native to tropical southeastern Asia. It is common in mixed deciduous forests of India, ascending up to 1500M. Geographical distribution is in Bangladesh, China, Malaysia, Pakistan, Srilanka and India. It is found throughout India from the sea-coast districts to the hill slopes as well (Singh, 1982). It is predominately wild but cultivated in Uttar Pradesh and Madhya Pradesh. It is a perennial tree in the tropics, but it can survive the unusually cold winter weather in its natural habitat and can recover from cold injury. Mature trees can also tolerate 46°C temperature in the summer season (Singh and Singh, 1987).

Possible locations are deciduous forests, like the forest in Mayurbhanj area of Orissa. This luxuriant tree is often grown in garden and home yards and also cultivated in botanical gardens.

### 2.5 Micropropagation- Historical Resume

Clonal propagation *in vitro* is called micropropagation. Clonal propagation is the multiplication of genetically identical individuals by asexual reproduction. Plant tissue culture involves asexual methods of propagation, which is meant for plant improvement. The term 'Plant tissue culture' broadly refers to the *in vitro* cultivation of plants, seeds, plant parts like tissues, organs, embryos, single cells, protoplasts, etc on nutrient medium under aseptic conditions.

In 1902, German plant physiologist, Gottlieb Haberlandt attempted to cultivate plant tissue culture cells *in vitro*. He is regarded as the father of plant tissue culture.
The concept of tissue culture arose with the phenomenon of totipotency followed by discovery of White’s (1934) medium for culturing roots followed by Murashige and Skoog’s (1962) completely defined medium for tobacco callus culture. With the Skoog and Miller’s (1957) hypothesis that an exact balance of auxins and cytokinins can promote differentiation and dedifferentiation of tissues, this advanced technology of cell and tissue culture has been developed and protocols for a variety of plant species have been made available. Micropropagation is of immense importance due to efficiency in multiplication and genetic stability.

2.5.1 Micropropagation through Plant Tissue Culture- A Review

Plant tissue culture has been used to solve various problems in biotechnological active compounds, storage of plant cells and organs, genetic engineering for improvement of important medicinal plants. Most research studies have been done with production and biotransformation of pharmacologically active principles by cell and tissue cultures. A number of reviews have been published (Butcher and Connolly, 1971; Staba, 1980; Barg and Ellis, 1981; Bajaj et al., 1998; Schumaker, 1991; Pattnaik and Debata, 1996; Arakiaswamy and Ignasimuth, 1998; Samarjeewa et al., 1993; Patil and Jayanath, 1997; Nadeem et al., 2000; Thakur et al., 1998; Priyadarashi and Sen, 1992; Banerjee et al., 1999; Nayak, 2000; Mathur et al., 1998; Ramawat and Nandawani, 1995; Arasu, 2004). A list of important general and medicinal plants where micropropagation has been successfully studied is presented in Annexure-4.

The successful cultivation for prolonged periods of cambial tissues of carrot root (Gautheret, 1939), tobacco (White, 1939) and carrot (Nobecourt, 1939) are the first true plant tissue cultures in the strict sense of prolonged cultures of unorganized materials. The work of Miller et al. (1995) on bud formation from cultured pith explants of tobacco
led to the discovery of kinetin. Steward et al. (1952) initiated work on cultured carrot explants and used coconut milk as a nutrient. Muir (1953) reported if the fragments of callus of *Tagetes erecta* and *Nicotiana tabacum* are transferred to liquid culture medium and the medium is agitated on reciprocal shaker, then the callus fragments break up to give a suspension of single cells and cell aggregates. An important technique of cloning large number of single cells of higher plants was developed by Bergman (1960). Morel and Martin (1952) for the first time recovered virus-free *Dahlia* plants. Morel (1960) also realized the potential of this method for rapid propagation of virus free orchids. The most universally used high-salt medium was developed by Skoog and his students (Murashige and Skoog, 1962). In addition to mineral salts, media contain an energy source, vitamins and growth regulators.

Micropropagation through Tissue Culture Biotechnology has become an important tool for research (Vasil, 1985). Clonal propagation of plants having medicinal value and production of Secondary metabolites from them are now increasing every year, (Miura and Hirata, 1998). *In vitro* propagation is an important tool for rapid multiplication of medicinal plants (Jha and Sen, 1984) as well as forest trees (Ahuja, 1991; Shankar and Mohan Ram, 1990).

*In vitro* clonal propagation gained momentum when Murashige (1974) gave the concept of development stages by defining three stages viz.

Stage I: Establishment,

Stage II: Proliferation,

Stage III: Rooting and hardening.

According to Debergh and Maene (1981), there are also three stages for micropropagation.

Stage I: Preparation of stock plants under hygienic conditions.
Stage II: Establishment of aseptic culture.

Stage III: Induction of meristematic centres and their development into rapid proliferating buds.

Stage III (a): Elongation of buds to form shoots and the preparation of uniform shoots
Stage III (b): Rooting, initial growth of produced shoots in a soil less potting mixtures.

2.5.2 Micropropagation of medicinally important plant: Steps and Techniques


They are described below.

2.5.2.1 Meristem/shoot tip/ axillary bud culture

This includes culture of meristems i.e. shoot apices and axillary buds in stem node segments. The plantlets produced are generally identical with parent plants. This method is commonly used in large-scale commercial propagation of important commercial plants like *Tinospora cordifolia* (Raghu *et al.*, 2006) and *Dendrobium candidum* (Shiau *et al.*, 2005).

Georges Morel (1965) was the pioneer in applying shoot tip culture for micropropagation of orchid *Cymbidium*, Kokate (1995) reported the inherent ability of a large number of medicinal plants to generate multiple shoots in culture of axillary and shoot tip meristems which opened up new avenues for production of phytopharmaceuticals, preservation and propagation of elite genotypes. Shoot meristem cultures have been utilized in a number of medicinal plants like *Dioscorea* (Heble and Staba, 1980), *Atropa* (Staba and Chung, 1981) and *Withania* (Heble, 1985), *Penthorum*
Chinese (Cao et al., 2007), *Morus alba* (Anis et al., 2003), *Ilex domusa* (Luna et al., 2003, *Clitoria ternatea* (Barik et al., 2007), *Muraya koenigii* (Bhuyan et al., 1997), *Vitex* (Usha et al., 2007). However, woody medicinal plants present a slightly different result. Norton and Norton (1986) reported that shoot multiplication in woody species of the family Rosaceae initially increased and then declined over a period of nine subcultures. Regarding this context a recent finding is that explants from mature trees can be made to produce multiple shoots but only after three subcultures (Mao et al., 2000).


Meristem culture has been useful for studying cultural variation in production of phytochemicals. Stojakowska & Kisiel (1997) observed in shoot culture from nodal explants of *Tanacetum sp* that the production of parthenolide was influenced by the genotype of the parent plant. Establishment of nodal cultures of *Artemisia annua*, produced an anti-malarial drug artemisin, has been reported but the cultures show lower levels of the chemical compared with the plant. However, supplementing the medium with GA3, casein hydrolysate and the chemical, naftifine in appropriate concentrations can raise the drug production to a higher level. Hence, *in vitro* shoot culture could be a viable alternative for the production of this drug (Fulzela et al., 1991).

### 2.5.2.2 Cell, callus and suspension culture

In this method living plant tissues derived from any part of the plant like cotyledon, hypocotyls, epicotyls, cotyledonary node, leaf segments, inter node segments or root segments are induced to grow in culture in an unorganized fashion and later
subjected to organic regeneration to produce plantlets. Chromosomal instability occurs often, especially on repeated subculture of the callus tissue prior to organogenesis and plantlet formation. It is useful in mutation programmes as in case of *Curcuma* by Nadgauda *et al.* (1978), *Sizigium cumini* by Remashree *et al.* (2003) and Banerjee *et al.* (1999).

Plant regeneration from callus might be useful in introducing new genetic variability to select useful somaclonal variants (D’Amato, 1985) in medicinal plants like *Aloe*, where genetic variation is limited due to continuous clonal propagation.

In *Catharanthus roseus*, the chemical sodium dikegulac (DK) was found to be effective in enhancing both callus biomass and also alkaloid content (Choudhury and Gupta, 1999). A simple protocol for high frequency regeneration via organogenesis has been standardized for sandal wood tree *Santalum album* (Sarangi *et al.*, 1999). In *Centella asiatica*, the rate of shoot bud regeneration was higher in calli from leaf-explant than stem, when subcultured in media containing BA, kinetin, NAA and adenine sulphate (Patra *et al.*, 1998).

Ramesh and Padhya (1990) obtained adventitious shoot from callus on excised leaf discs of *Azadirachta indica*, wood and Braun’s medium supplemented with BA and kinetin showed best response. In callus culture of *Glycorrhiza glabra*, calli can be maintained in appropriate cultured conditions (e.g. by replacing 2,4-D by NAA) reported by Henry *et al.* (1991).

*In vitro* production of alkaloids in medicinal plant *Atropa* was noted in root callus, but not in stem and leaf calli (West and Mika, 1957). In 1963, a preliminary work in China was reported on the callus culture of medicinal plants like *Panax ginseng* and *Datura* in an attempt to produce ginseng, saponin, atropine, etc. (Shei-Wei, 1978). An important observation by Fuzi (1991) was in different *Podophyllum* species.
Podophytotoxin content of leaves of regenerated plants was three times higher than those of original plants, but roots of the tissue culture plants showed only one-fourth during to the roots of mother plants. Thus an organ-specific variation is suggested.

Several callus tissues of *Atropa belladona* having low or even no alkaloid production in the beginning of the cultivation showed increasing production later i.e. seventh to ninth passages of tissue culture (Bajaj and Simola, 1991).

2.5.2.3 Organogenesis

Organogenesis involves *in vitro* propagation by organ formation. Adventitious or axillary shoots are induced *in vitro* and these are subsequently rooted. Somatic embryogenesis of many woody species was reported by Ahuja (1993). Joshi *et al.* (1991) observed multiple shoot formation from excised seedling segments of *Anogeissus pendula* through indirect organogenesis. Cotyledon segments were noticed to have a higher organogenic potential than epicotyls segments, when cultured in MS basal media supplemented with 1.0mg/l and 0.1 mg/dl IAA. Shoot organogenesis on hypocotyls example in *Psidium guajava* was by Singh *et al.* (2002) where activated charcoal was used for better response in rooting.

Various culture media are used for organogenesis. The most widely used media are MS (Murashige and Skoog, 1962) White (1963), Gamborg (1968) and SH (Schenk and Hildebrandt, 1972). Skoog and Miller (1957) first indicated the role of media in morphogenesis of *Daucus carota*.

2.3.2.4 Somatic embryogenesis

Somatic embryogenesis is the process by which the somatic cells or tissues develop into differentiated embryos and each fully developed embryo is capable of developing into an independent plantlet. It is the most promising method but it is applicable to a relatively fewer species. Various important plants like *Panax ginseng*
(Tang, 2000), *Santalum album* (Das et al., 2001), *Safed musli* (Purohit et al., 1994), *Clitoria ternatea* (Dhanalakshmi and Lakshmanan, 1992) and *Zingiber* (Kacker et al., 1993) have been propagated by this method. For genetic manipulation, this technique has a lot of significance. The initiation and development of embryos form somatic tissues in plant culture was first recognized by Steward et al. (1958) and Reinert (1958, 1959) in cultures of *Daucus carota*. Sharp et al. (1980) described two routes to somatic embryogenesis: 1) direct embryogenesis i.e., embryo initiate directly from the explants tissue and 2) indirect embryogenesis i.e. callus from explants takes place from which embryos are developed.

Somatic embryogenesis has been achieved in a number of plant species. Rout et al. (1995) reported somatic embryogenesis in *Acacia catechu*. They observed that woody plant medium supplemented with 13.9 μM Kinetin and 2.7 μM NAA was most effective in producting somatic embryos. The further reported that addition of 0.9-3.5 μM L-proline enhanced the formation of somatic embryos.

Gill et al. (1995) developed protocol for somatic embryogenesis in *Citrus reticulata*, embryogenic calli was initiated from cotyledon, epicotyls and root segments. Murthy and Saxena (1998) reported somatic embryogenesis in *Azadirachta indica* using mature seeds. It was reported that regeneration occurred via somatic embryogenesis, direct embryo formation and through an intermediary callus phase when the seeds were cultured on to MS media supplemented with thidiazuron (TDZ) in connection between 1-50 μl.

Somatic embryogenesis in *Dalbergia sissoo* using immature zygotic embryos from MS medium supplemented with 0.46-1.16 μM Kn and 6.78-9.08 μm 2,4-D by Das et al. (1997).
2.5.3 Cryopreservation

Cryopreservation techniques show promise in a number of medicinal plants like *Lavandula* (Watanable *et al.*, 1983) and *Atropa belladonna* (Bajaj, 1998; Bajaj and Simola, 1991). According to Bajaj and Simola (1991), reported that cryopreservation of *in vitro* cultures of medicinal and alkaloid producing plants is an area of tremendous interest from retention of biosynthetic potential in cell cultures, genetic stability in high yielding somaclones and conservation of germplasm.

2.3.4 Shoot multiplication and shoot elongation

Cytokinins are usually required during the multiplication step. The tendency of axillary shoots to develop on shoot depends on the genotype, the type of shoot and the number of previous subcultures (Bergman *et al.*, 1984). Sometimes higher concentration of cytokinins gives rise to suppressed growth and bushy like appearance. By lowering the concentration of cytokinins is usually required for shoot multiplication and elongation.

2.5.5 Rooting

Treatment of shoots with exogenous auxins, preferably synthetic compounds such as IBA (Indole butyric acid), IAA (Indole acetic acid) and NAA (a-Naphthalene acetic acid) stimulates root initiation (Gasper and Coumans, 1987).

2.5.6 Acclimatization

In *vitro* cultured plantlets are generally grown under high humidity and low light conditions. Plantlets removed from cultures are very susceptible to wilting, desiccation and infection. The humidity during the period immediately following transplant must therefore be carefully controlled, especially in hardwoods. Gradual acclimatization periods with decreasing humidity are necessary for plants to survive the transition from culture vessels to green house and field conditions (Sommer *et al.*, 1986)
2.5.7 Source of explants and its nature for establishment of culture

Cloning in vitro and in vivo is adversely affected by maturation and the resulting phenomenon of reduced growth rate and reduced rooting ability. Maturation is a major barrier in tree species for tissue culture. Franclet et al. (1987) reported that these effects vary with species, genotype and age of the plant. Skirvin (1980) observed that the type of the explants varies with each plant species and the most suitable one should be determined for each species.

Norton and Norton (1986) reported that the explants length (2.5-20mm) of axillary bud in Prunus and Spirea resulted that explants taken from the top of the canopy produced most shoots. If the explants size is small, there will be difficulty in survival of the explants (Hussey, 1978).

It is usually more difficult to establish shoot cultures from mature trees than from juvenile plants (Bonga, 1987; Hackett, 1987), if the species is more juvenile, it is easier to propagate vegetatively. Explants from the lower branches of the crown are normally easier to establish than explants from more mature tissues in the upper part of the crown (Bonga, 1987). Also stem sprouts are a favourable juvenile alternative (Chalupa, 1984; Mascarenhas et al., 1982; Franclet et al., 1987). However, Azadirachta indica (Yaseen, 1994), Dalbergia sissoo (Chauhan et al., 1994), Tectona grandis (Gupta et al., 1980) and Artocarpus heterophylus (Amin & Jaiswal, 1993) have been cloned in vitro successfully taking nodal and terminal bud explants from mature trees.

Season is also a very important factor for mature tree tissue culture. Spring (March-April) was the best season to initiate tissue culture from mature trees. The best seasons for bud initiation of most plants are spring, coinciding with bud break in late summer (Bonga, 1987; Welander, 1983). However, consideration has to be taken to the risk of contamination, which also is season dependant (Bonga, 1987). Amin and Jaiswal (1993)
reported that November to January was the best season for initiation of cultures from field grown trees of *Artocarpus heterophylus*.

Oxidation of poly-phenols is extremely abundant in woody plants. Because of the poly-phenols the explants gradually turn brown and ultimately die. Crompton and Preece (1986) have vividly described methods to overcome the problems associated with phenol exudation during explants establishment i.e. pretreatment like treatment with antioxidant compound such as citric acid, ascorbic acid, polyvinyl pyrrolidone etc. and supplementation of activated charcoal and antioxidants in the culture media are recommended.

2.5.8 Culture media for tissue culture

The MS medium of Murashige and Skoog (1962) salt composition is very widely used in different culture systems. The B5 medium of Gamborg *et al.* (1968) or its derivatives have been useful for cell and protoplast culture. It differs from MS medium in having much lower amounts of nitrate in the form of ammonium. The N₅ medium was developed for cereal anther culture and is used successfully with other types of cereal tissue culture (Chu, 1978). All the tissue culture media contains the ingredients like macronutrients, micronutrients as carbon source, vitamins and growth regulators.

2.5.8.1 Macronutrients

In macronutrients the major nutrients are Nitrogen, Phosphorous, Potassium, Calcium and Magnesium. Nitrogen has a greater significance for the growth and development of the plant. The form and amount of nitrogen in the *in vitro* medium have significant effects on growth and differentiation (Thorpe, 1983). Most media incorporate nitrate and ammonium salt as nitrogen source for growth (Gamborg & Skyluk, 1981). Specific forms of organic nitrogen, which are readily assimilated by plant cells and have growth promoting activities, include amino acids, urea and polyamine etc.
2.5.8.2 Micronutrients

It is quite essential to have the elements like iron, manganese, zinc, boron, copper and molybdenum but lack of any one of these elements caused metabolic disorders if not corrected in time (Rao et al., 1987).

Iron helps in the nutrition of plants. Chlorophyll and enzymatic activities are influenced by the application of iron (Agarwal and Srivastava, 1984; Singh et al., 1994). Copper plays an important role in reorganization of quinine like substances and thus promotes photosynthesis. Copper deficiency is best seen in deciduous trees, herbaceous legumes and cereals (Rao et al., 1987). Zinc serves as an activator of several enzymes. Zinc deficiency in *Pinus* leads to dieback of young shoots, reduction in length of terminal needles, resetting and yellowing as reported by Hill and Lambert (1981). Manganese serves as an activator for enzymes connected with reduction of nitrate, an important step in nitrogen metabolism of plants (Rao et al., 1987). Role of Boron in the control of auxin metabolism has also been suggested by Pollard et al. (1977). Most tissue culture media contains Molybdenum at concentration 1 M and 0.5 μm chlorine. Chlorine deficiency leads to chlorosis, reduced leaf growth, wilting, necrosis of apical leaflets and stunted club shaped roots (Hewitt and Smith, 1974).

2.5.8.2.1 Carbon source

Sucrose or glucose at 2-4% is suitable carbon sources which are added to the basal medium. Fructose, maltose and other sugars also support the growth of various plant cells. The sugar of transport in intact plant is generally the best carbon source (Thorpe, 1982).

2.5.8.2.2 Vitamins

Vitamins like Thiamine (B₁), Nicotinic acid (B₃) and Pyridoxine (B₆) are most frequently used in the media. Of these, thiamine is the basic vitamin required by all cells.
and tissues. Addition to these, other vitamins are also required. Gupta et al. (1980) reported that the addition of biotin and calcium pentothenate (B₅) to MS medium was essential for promoting optimum growth in Eucalyptus cultures. Myo-inositol is another vitamin whose presence in small amount is beneficial.

2.5.8.3 Plant growth regulators

Plant growth regulators include auxins, cytokinins, gibberellins, abscisic acid and ethylene. Each type of growth regulator has a wide range of physiological effects in different plants. The discovery of auxins (Went, 1926) followed by the discovery of cytokinins (Miller et al., 1955) as regulators of cell division paved the way for the rapid regeneration of plant tissue culture. Skoog and Miller (1957) reported the relative concentration of auxins and cytokinins controlled the morphogenetic response of tobacco tissue culture.

2.5.8.3.1 Auxins

Auxins like IAA, IBA, 2, 4-D (2,4-Dichlorophenoxyacetic acid) and NAA are commonly used. Indole-3 acetic acid (IAA) was the first substance to be recognized as an auxin. Association of this growth regulator performs cell enlargement and its involvement in morphogenetic processes such as root formation in many species is widely known (Thorpe, 1978). Roots were induced in several forest species like Tectona grandis (Gupta et al., 1980) and Santalum album (Bapat and Rao, 1979). Indole-3-Butyric acid (IBA) was used to induce roots on shoots derived from callus of Eucalyptus citriodova, roots on in vitro shootlets of Gmelina arborea was reported by Thirunavoukkarasu and Debata (1998). α-Naphthalene acetic acid (NAA) was also used with cytokinin for obtaining shootles by Remashree et al. (2007) in Syzygium cumini.
2.5.8.3.2 Cytokinins

Cytokinins are mainly concerned with cell division, modification of apical dominance and shoot differentiation in tissue culture. Rapid progress in aseptic culture technology occurred after the discovery of Kinetin (Miller et al., 1995). It is widely used for shoot bud induction. Another important hormone is (BA) 6-Benzylaminopurine (BA) which is more effective than Kinetin. It is stable, highly effective and inexpensive. Read et al., (1982) obtained multiple shoot formation on Alnus glutinosa when grown on medium containing 1mg/l of BA.

2.3.8.3.3 Gibberellins

Gibberellin promotes the growth of the cell cultures at low density and enhances callus growth. It decreased the shoot formation but have strong effect on elongation of plantlets. Sankhla et al. (1993) reported that gibberellins biosynthesis inhibitors increased shoot formation in Albizzia julibrissin. Abscisic acid is very rarely used in the culture medium either to stimulate or inhibit the callus growth depending on the species (Ranjam, 1993).