

## 1.1 AYURVEDA

The term Ayurveda, derived from Sanskrit word means knowledge/science (Veda) of life (Ayur). After being transmitted orally for thousands of years, the ancient Ayurvedic texts finally were written and preserved in Sanskrit (**Rao, 1985**). Founded on the collective wisdom of ancient Hindu saints and healers, Ayurveda grew into a medical science. Ayurveda refers, essentially and basically to promote the health. The Ayurvedic text, an off-spring of the Atharvaveda, appeared sometime between 1500 to 1000 BC described two schools of learning - physicians (Atreya) and surgeons (Dhanvantari) and eight branches of clinical science i.e. internal medicine (Kayachikitsa), surgery (Shalyatantra), pediatrics (Balachikitsa), toxicology (Damstrachikitsa), psychiatry (Grahachikitsa), ophthalmology (Shalakyatantra) and otorhinolaryngology (Urdhvangachikitsa), rejuvenation (Rasayana), and sexual vitality (Vajikarana).

Ayurveda often has been considered as one of the earliest medicinal system of the world (**Sharma, 1995**). Charaka and Sushruta Samhita and Ashtanga Hri-daya (600 AD) are the three most revered ancient Ayurvedic texts (**Sharma, 1994**). Charaka and Sushruta are considered to be the fore-fathers of the system. In an attempt to simplify the voluminous Charaka and Sushruta Samhita, many Ayurvedic texts such as Vagbhata (700 AD), Madhav Nidan (**Sharma, 2000**) Sharangdhara Samhita, and Bhava Prakash (1600 AD) have emerged. Centuries later, these ancient texts still are available, although much has been lost to the change of time and altered human perceptions and translations.

### 1.1.1 General concept and fundamental principles

The Ayurvedic philosophy (Vaisheshika) describes a unifying hypothesis linking the universe with all living and nonliving matters where human beings and plant in the hierarchy of cosmic evolution consist of the same basic matter (Panchbootas i.e., earth, water, air, fire

and ether). Consciousness or intelligence (Sattva), motion or action (Rajas) and the inertia that resists them (Tamas) are the three omnipresent nonmaterial qualities (Gunas) that govern all material forms of basic matter. These material and non-material attributes subsequently dictate the medicinal and healing properties to the plants. According to Ayurveda the human body is a replica of the universe where the basic matter gives birth to three life forces or energy called Dosha in all existing matters (**Sharma, 1995; Ranade, 1996**).

As per Ayurveda the diversity in the universe is due to the transformation of the earth matter (as a Panchbootas) on contact with the fire energy. Similarly, all anabolic and catabolic transformations in the body occur as a result of the existence of the inherent fire (Agni) energy. The manifest world is traced, however, to the unmanifest called Prakruti, which signifies nature and individual human constitution and is driven by a harmonious equilibrium between cosmic intelligence, ego (Ahamkara) and self-consciousness (**Svoboda, 1996**). The practice of Ayurveda in daily life aims at maintaining the harmony to ensure optimal health. However the over-all Prakruti comprising of physical, psychologic, and functional (Dosha) attributes remains unchanged for life.

The three Doshas (Vata, Pitta, and Kapha) are the primary dynamic physiologic forces that govern the biomotor, metabolic, and preservative (homeostasis) activity. Dosha are formed constantly in the body from food and other physiologic processes. Vata (air-ether), is the most powerful force that governs motion and controls cell division, arrangement and differentiation; impulse transmission (including cardio-respiratory and all the higher functions in the brain); movement of body fluids and excreta and parturition and it is most relevant to the nervous and musculo-skeletal system. Pitta (fire) governing metabolism and the formation of tissues and waste products; is most relevant to the digestive and endocrine system. Kapha (earth-water) increases cell mass, promote bonding of tissues, prevent destruction of tissues, maintain strength and immunity, and determine body growth.

Each Dosha has its own characteristic anatomic, physiologic, and psychologic expressions. Vata is dry, cold, light, and clear. Pitta is hot, light, fluid, and clear. Kapha is cold, wet, heavy, cloudy, and static. The three Dosha are in equilibrium (Samya) with respect to their quantity (Pramana), quality (Gunas), and functions (Karmas) and remain in balance forming the basis of health (Arogya). The body adjusts to the numerous changes that continuously occur in Dosha under the influence of numerous factors (e.g., diet, seasons, activity and age). Although Vata, Pitta, and Kapha often colonize in the intestine, stomach and chest, they are ubiquitous. The gastrointestinal system plays a central role for the health of every other system. There is a digestive fire (Agni) in the intestine that converts food into a homogeneous mass, tissues (for assimilation and metabolism and tissue formation), and certain elements (for special senses). The Dosha equilibrium is essential for optimal health and prevention of disease and any imbalance causes the disease (**Sharma, 1995; Ranade, 1996; Upadhyay, 1998**).

Each individual is composed of seven constituents (Dhatu): plasma (Rasas), blood, muscle and flesh, bone, marrow, fat, and semen/ova (Shukara). Waste products (Malas) can be gross (e.g., urine, feces) or subtle (e.g., epithelial linings of the eye). Rasayana aims at strengthening of Rasa. Fluids and life forces travel through channels (Srotas) that connect tissues with one another and the gut.

The body strength (Bala) has two components - physical strength and resistance to diseases (Vyadhikshamatwa). The immunity depends on the quality of tissues (especially Rasa), Kapha, Shukara, and Ojas. Ojas, an end product of sequential tissue metabolism, determines body energy, strength, and immunity and is strengthened by Rasayana. Excess sexual activity diminishes Ojas. The immunity can be natural or temporal (related to age and seasons) and is developed through good life practices, including diet, sleep, and rejuvenation therapy.

Ayurveda as a holistic science is customized to the individual's Prakruti that promotes the practice of rejuvenation (Rasayana) and virilification (Vajikarana) in daily life (**Ranade, 1996**).

### **1.1.2 Validation and modern relevance**

Ayurveda must adapt to the modern human without losing its core strength and relevance. Recent controversies, probably more political than science, regarding the current status and recognition of Ayurveda in the United Kingdom have been addressed seriously by a lucid historical review (**Vaidya, 2001**) on the Ayurvedic contributions to the world of medicine. The human genome study has revealed that each human being has different genomic condition and thus each individual is different from another. This concept exactly the same as mentioned in Ayurveda as Prakruti. Naturally and scientifically two persons differing in Prakruti can not be treated with same formulation. In the light of this concept only Ayurveda since time immemorial has advocated for specific treatment to specific person depending on the Prakruti. Scholars have begun to ponder the possibility of futuristic designer drugs customized to the patient's illness and based on an Ayurvedic database; matching an individual's diagnosis (based on Dosha-prakriti and other etiologic factors as perceived in Ayurveda) and prescribing the treatment accordingly herbal-mineral pharmacopoeia (**Patwardhan, 2000**).

To a reasonable extent, centuries of clinical use, however anecdotal, promise inherent safety and the drug trials referred to a large extent seem to confirm that view. Despite popular belief, although, Ayurvedic medicines may have toxicity (**Bhatt AD and Bhatt NS, 1996; Luiz, 1998; Chopra, 2000**) but observations would suggest that toxicity is less in frequency and intensity compared to modern medicine. In modern era Ayurveda faces the challenging tasks of drug standardization (especially when multicomponent) and validation (clinical and structure-activity relationships). Despite socioeconomic and logistic problems

Ayurvedic drugs in popular use today can be evaluated rapidly in controlled clinical situations.

## **1.2 RASA SHASTRA**

Rasa Shastra which can be named as the Ayurvedic alchemy (science of metals) is an important branch of Ayurvedic pharmacology. This branch deals with the use of metals, minerals, gemstones and poisons and their processing to produce highly valued therapeutic formulations, which helps in combating acute conditions or serious diseases. In the ancient Ayurveda the emphasis has been over usage of the herbs however, later on the animal products, metals and minerals started to find favor in the Ayurveda as it was realized that the minerals and metals are very effective and potent for immunity, rejuvenation and for the elimination of diseases. It was also found that the parada (mercury) is the most important ingredient of the Rasa Shastra (**Puri, 2003**).

### **1.2.1 History and Origin of Rasa Shastra**

Rasa Shastra or 'Vedic Chemistry' is an off-shoot of Ayurveda was developed around the period of Buddha around 2500 years ago. Derived from Sanskrit, Rasa has several meaning attached to it - "Rasyate aaswadyate iti Rasa" which stands for taste, "Rasati shariire aasu prsarati iti Rasa" meaning juice, "Rasati aharahargachhati iti Rasa" that stands for the first material formed after digestion or liquids, under the transportation system of the body like plasma, lymph and "Rasanaat Sarva dhatuunam Rasaityabhdhiiyate" is for material which is capable of lick and digest all metals (**Sharma, 1981**).

The origin of Rasa Shastra in the Ayurveda has its roots in the alchemy or the science of the metals. Etymologically the Rasa Shastra is derived from the words Rasa (mercury) and Shastra (literature). In ancient times Rasa Shastra was a process of the extraction of precious metals like gold and silver from mercury. The experts applied the same analogy to the human body and found that the body dhatus can also be

enriched in the same way by the use of different metals. This study came to be known as the dehavad. The study of dehavad and the use of metals were successful and it was found that the mercury was very useful and effective when compared to its herbal counterparts and moreover it was effective in very small quantities (**Shastry, 1999**).

As per mythology the Lord Shiva imparted the initial knowledge of Rasa Shastra. In earlier days, Rasa chiktasa was used for achieving immortality however later it was utilized for the treatments and subsequently Indian Ayurveda made an extensive use of Rasa Shastra so much so that it became the vital or inseparable component of the therapeutic process of Ayurveda (**Murthy, 1993**).

Rasa Shastra can be described as Ayurvedic pharmaceuticals, which deals with the drugs of mineral origin, their varieties, characteristics, processing techniques, properties and their therapeutic uses. There are two main categories of Rasa Shastra - Alchemy and Rasayana. While alchemy is involved in turning mercury into gold, Rasayana, on the other hand, helps in the rejuvenation of the mind and body. This science is often referred to as 'alchemy' and the resultant medications are called Rasas, which mainly comprise of metallic ashes called bhasmas. These bhasmas, or lighter forms of metals, are processed as organo-metallics that work as carriers (yogavahi). This means they are able to carry the herbs mixed with them faster to the desired site and start the therapeutic action immediately (**Ghanekar, 1981**).

### **1.2.2 Advantages of the Rasa Shastra**

In Ayurveda, the Rasa Shastra has been considered more useful and effective compared to the herbal therapy. It is said that the Rasa Shastra has immense therapeutic applications some of which are prevention of ageing and reduction in age-related disorders (**Sharma, 1983**). Some other features that make Rasa Shastra more advantageous than the other form of preparations are:

- a. They work in smaller doses and are faster in action, making it the therapy of choice.
- b. The assimilation of Rasa preparations in the body is much faster because the preparation undergoes different processes called sanskaras. This helps in active and quick assimilation of even the minute doses in the body.
- c. Incinerated minerals used in Rasa Shastra possess excellent therapeutic values and they have a longer shelf life.
- d. The efficacies of the Rasa preparation do not alter with time, which means that the Rasa formulations have no expiry date.
- e. The minerals are persistently available compared to herbs, which are mostly seasonal.
- f. The preservation of Rasa formulations is easy and less cumbersome.
- g. The taste of Rasa medicines are generally neutral and are less cumbersome to administer.

### **1.2.3 Importance of Rasa Shastra in Ayurveda**

The modern living is prone to certain side effects of the developmental process. The diseases related to pollution, radiation and stress are on the rise. Improper lifestyle and the imbalanced diet along with the consumption of junk and chemically processed food further aggravate the problem. Although modern medicine deals with these diseases through the heavy usage of the steroids, the continuous use of steroid and the lifestyle related discrepancies make these diseases chronic in nature and decrease the immunity levels of the patients (**Diwedi, 1992**). It has been observed that the herbal preparations alone are not capable to deal with the complicated and chronic nature of diseases. That is the reason why the Rasa Shastra has been accorded so much importance in the Ayurveda. The faster action of formulation of Rasa Shastra is considered more appropriate to deal with the above-mentioned complications and justify the integration of the Rasa Shastra in the Ayurvedic therapeutics (**Puri, 2003**).

### 1.2.4 Rasas

After classifying mercury as the Rasa (after which this branch has been named), the ancient chemists classified the other metals, minerals and gems into dhatu, upadhatu, ratna, upratna, maharasa, uprasa and sudha varga. Mercury, when properly processed/prepared, is known not only to balance all the three doshas, but also to impart a soothing effect to the body, gives a firm physique, a stable mind and avert the diseases and old age. Mercury nurtures the body parts and also enhances the strength of the eyes. It is used as vrisya (aphrodisiac), balya (tonic), rasayana (rejuvenative), vrana sodhana and ropana (wound cleaner and healer), and krimighna (antimicrobial) (**Rasaratnasamuchaya, 2003**).

Similar to herbs minerals also have their own nature and characteristics e.g gold is madhura (sweet) and kasaya (astringent) in rasa, snigdha (oily) and laghu (light) in guna, sita (cold) in virya, and madhura (sweet) in vipak. It acts as an antimicrobial and antipyretic, and improves body complexion and control the wastes of the body tissues. Silver is kasaya (astringent) and amla (sour) and it is known to give strength to the brain, heart and stomach.

In consideration of the importance of elements in body functions the metal-based formulations have been in use since time immemorial and Rigveda mentions the use of gold, silver, copper and bronze in the treatment of diseases. Nagarjuna, the father of Rasa Shastra, endeavoured to free the entire world from diseases by using processed mercury. According to the Rasa Shastra the metals, referred to as Dhatu and Updhatu, supplement several essential, disease-preventing elements in the tissues of human body. The metal-based formulations are particularly effective in curing diseases related to organs where such metals are naturally present and perform their essential role (**Rasatarangini, 1979**).

The quality of a substance or drug is represented by its Rasa. Dravyas are classified into six categories according to the six Rasas.

- a. Madhura Rasa (sweet): Sweet taste promotes handsomeness and longevity and tones up the Ojas (vitality). It helps restore the vitiated humor Pitta and alleviates morbid Vata humour.
- b. Amla Rasa (acidic): It stimulates the salivary glands and promotes appetite and digestion. It provokes the humors - Kapha and Pitta.
- c. Lavana Rasa (saline): It enhances the flavour of food and acts as a carminative and laxative and allays Vata humor.
- d. Katu Rasa (pungent or acrid): It acts as an anthelmintic, dilates the body channels and allays the Kapha humor.
- e. Tikta Rasa (bitter): Apart from parasitocidal and antipyretic, it is also a carminative.
- f. Kashaya Rasa (astringent): It soothes the Kapha and Pitta humors in their irritated state.

**Table 1.1 Different Rasa formulations of Ayurveda (Rasatarangini, 1979; Rasaratnasamuchaya, 2003; AFI, 2003)**

S. No	Rasa formulations	Therapeutic uses
1	Agnikumar Ras	Antidysenteric, aphrodisiac and tonic. Indicated in acute diarrhoea, dysentery and chronic fever.
2	Agnisandipan Ras	Aperients and sedative, indicated in indigestion, dyspepsia, stomach pain and gastric trouble etc.
3	Amvatari Ras	Alterative, antirheumatic and diuretic. Indicated in body pain and pain in joints.
4	Anand Bhairav Ras	Diaphoretic, antiperiodic and diaphoretic. Indicated in cough and cold, fever and other respiratory disorders.
5	Arshakuthar Ras	Alterative tonic and laxative. Used in irregularity of bowels, constipation and piles.
6	Bangeshwar Ras	Antacid, diuretic, and urinary antiseptic, indicated in urinary disorders.
7	Basant Kusumakar Ras	Alterative tonic. Used in debility of heart and brain etc.
8	Bhuwaneshwar Ras	Alterative, Stomachic, antispasmodic & stimulant. Indicated in dysentery, indigestion, flatulence etc

9	Chandrakanta Ras	Analgesic and alterative, indicated in hemicrania and other associated symptoms.
10	Chintamani Ras	Cardiac tonic, alterative & haematinic. Used in heart diseases, congestion, cardiac failure and dilation of heart.
11	Garbhpal Ras	Tonic, stomachic and alterative, used in nausea, vomiting, and similar ailments during pregnancy,
12	Ichchhabhedi Ras	Hydragogue and cathartic. Used in constipation, dropsy, paralysis and in all other conditions needing strong purgatives.
13	Jalodharani Ras	Specific remedy for Ascites (jododar). It cures dropsy and improves digestive power.
14	Jwarankush Ras	Alterative and febrifuge. Indicated in all sorts of fever, remittent and interremittent etc.
15	Kaminivindravan Ras	Alterative tonic and aphrodisiac, gives strength and vitality and thickens the semen. Useful in impotency and loss of semen,
16	Kasturibhushan Ras	Indicated in typhoid, pneumonia and high fever and delirium etc.
17	Krimikuthar Ras	Anthelmintic, carminative and antispasmodic. Used in worms, cholera, indigestion and whooping cough.
18	Laxmivilas Ras	Antipyretic, stomachic, and expectorant. Indicated in pneumonia, typhoid and delirium
19	Mahajwarankush Ras	Antiseptic and antipyretic. Used in malarial fever.
20	Nityanand Ras	Tonic and alterative. Used in filaria.
21	Pradantak Ras	Alterative tonic, diuretic and astringent. Indicated in leucorrhoea, anemic conditions and in urinary diseases.
22	Smriti-Sagar Ras	Nervine stimulant, alterative and tonic. Used in epilepsy, mania and weakness of memory.
23	Shwaskuthar Ras	Gastric stimulant, antispasmodic, diaphoretic and sedative. Used in asthma, allergy, fever, tonsillitis, painful neuralgic affections and as a restorative.
24	Vat Chintamani Ras	Alterative tonic and anti rheumatic. Used in heart disease.
25	Vat Gajankush Ras	Alterative, carminative and stomachic. Used in paraplegia.

The word 'Rasayana' literally means the path that 'Rasa' takes (Rasa: plasma; Ayana: path). Rasayana drugs act inside the human body by modulating the neuro-endocrino-immune systems and have been found to be a rich source of antioxidants (**Brahma and Debnath, 2003**). These Rasayana plants are said to possess the following properties: preventing ageing, re-establishing youth, strengthening life, brain power and preventing diseases (**Ghanekar, 1981; Sharma, 1983**). All of these virtues imply that Rasayana increase the resistance of the body against any onslaught.

'Rasayana' therapy is done for a particular period of time with strict regimen on diet and conduct. Rasayana drugs are rich in their antioxidant potential, are good hepatoprotective and immunomodulating agents. 'Rasayana' is a specialized procedure practiced in the form of rejuvenation recipes, dietary regimen and special health promoting right conduct and behavior, Rasayana drugs have been proven to treat epilepsy (**Singh and Murhty, 1989**), convulsive disorders (**Diwedi and Singh, 1992**) and to reduce anxiety, apprehension and keep the mind calm and cool (**Puri, 2003**).

As per Charak and Sushruta Samhita, the aims of Rasayana are to increase the body's resistance to disease (akin to immunity, called Vyadhi-kshamatva), increase life span, and promote intellect and strength. Scientists from Ayurveda and modern phytopharmacology are researching on ancient Rasayana plants for their potent antimicrobial, anticancer, antioxidant and immunomodulation potential (**Ziauddin, 1996; Dahanukar, 1997; Rege et al., 1999**). According to one hypothesis the Rasayana plants cause a non-specific strengthening of immune response and tolerance to antigenic challenges and promote positive health. As an example *Withania somnifera* (Aswagandha) (**Ghosal, 1989; Ziauddin et al., 1996; Begum, 1998**) compared with the ginseng predominantly reduces the Vata and Kapha Dosha.

Some other plants with well-known Rasayanic properties are *Emblica officinalis* (Amlaki), *Zingiber officinale* (Ginger), *Curcuma longa* (Curcumin), *Tinospora cordifolia* (Guduchi), *Allium sativum* (Garlic), *Asparagus racemosus* (Shatavari), *Terminalia chebula* (Haritaki), *Boerhavia diffusa* (Punarnava), *Centella asiatica* (Mandukaparni), *Crotalaria pluricaulis* (Shankapushpi), *Semecarpus anacardium* (Bhallatak), *Azadirachta indica* (Neem), *Vitex negundo* (Nirgundi), *Ocimum sanctum* (Tulasi), *Piper longum* (Pippali), and *Aloe vera* (Ghritkumari). Many of these plants are well known for their antiarthritic properties (**Chopra, 2000**). The fruits of *Terminalia chebula* is an excellent example of herbal medicine used to balance all three Dosha and finds use in curing diverse ailments. Owing to therapeutic potential in the Tibetan system, it is called the king of all medicines (**Frawley, 1994**).

### 1.2.5 Bhasmas

Use of metallic preparations (Bhasma), bhasmikarana process is to prepare the formulations, is unique to the Ayurveda. It is believed that the bhasmikarana process converts the metal into its specially desired chemical compound, which eliminating the toxicity of the metal and attaining/acquiring the necessary medicinal benefits (**Wadekar et al., 2005; Kumar et al., 2006**). According to Ayurveda provides qualitative tests to be performed to assess the efficacy of the bhasmikarana process, ensuring that the resulting drug is very fine (small grains) possess no metallic shine (**Patel, 1986; Svoboda, 1998**).

**Preparation of Bhasmas:** As per Ayurveda, preparation of Bhasmas is an elaborate process involving shodhana and bhasmikan. Where, metals are first purified through a process called shodhana, during which the metal is repeatedly heated and then cooled in herbal extracts. This is followed by bhasmikan where, the shodhit metal is repeatedly triturated with herbs (bhavana) and calcinated in closed earthen crucibles in a pit, by burning cow-dung cakes (a process called puta), to obtain Bhasma. The size of the pit, the number of cow dung cakes to be

used to obtain a specific temperature and the duration of heating are specified in detail. As this process is repeated in prescribed manner for each preparation, hence there are dasa puta (10 cycles), satha puta (100 cycles) and sahastra puta (1000 cycles), etc. To ensure that the Bhasma is properly prepared, a set of tests are specified.

Though Bhasma preparations are widely used in Ayurveda, practically nothing is known as to what happens to the metals when they are subjected to bhavana with herbs followed by subsequent calcinations process. The traditional texts also do not throw any light on the changes undergone by a metal during the above processes.

It is interesting to note that same metal is processed (i.e. given bhavana) with different sets of herbs, to be used for different indications. In this context, it is all the more interesting to study as to what changes the metal goes through during the different steps of bhavana and subsequently during the calcinations process that it acquires a non-toxic and therapeutically efficacious form. Bhasma are known to be effective in very small doses, usually a few milligrams, shelf-life of Bhasma is believed to be infinite.

Now newer technologies for analyzing the composition of drugs and formulations help answering the above questions, at least partly. Atomic absorption spectroscopy and flame photometry studies have their limitations as they only reveal the presence of the specific metal and the quantity of the metal in a preparation. Earlier, there used to be a view that metal oxides/sulphates and other salts are formed during the process of puta. However, a recent study showed that the formation of metal oxide is an intermediate change; and that the metal subsequently takes a new, hitherto unknown, form that still needs to be characterized (**Tripathi et al., 2003**).

With the advent of nanotechnology, the current belief is that during bhasmikaran the metal acquires small particle size, which is responsible for its enhanced bioavailability and activity and hence the

dose is less. Even if it is so, it remains yet to be answered as to why the same metal is given bhavana with different set of herbs to be to be effective in different disease conditions. There has to be much more to the elaborate process involved in the preparation of Bhasma than simply imparting micronization of the metal particles. If we understand this, we may be able to reduce the number of steps, especially for satha puta (100 cycles) and sahastra puta (1000 cycles), using modern technologies and equipments available, including grinding machines and muffle furnace to ash etc. It may also throw some light on the mechanism of action of Bhasma and open new avenues for understanding the disease and achieving cure using Bhasma.

Recently, doubts have been raised on the safety of the Ayurvedic preparations using metals and Bhasma prepared from them and concern is expressed regarding the metal toxicity of traditional preparations containing metal and Bhasmas. Although Ayurveda fraternity argues that toxicity can arise only from a metal when present in its free form, and that a Bhasma prepared according to the classical methods never contains a metal in free form and therefore if taken under proper supervision and guidance the question of toxicity does not arise, still the dispute arise need to be resolved. Ayurvedic practioners themselves admit that due to commercialization of Ayurvedic preparations, some of the manufacturers are resorting to short-cuts in preparing Bhasma, because of which there are chances of the metals being present in free form metal in the final preparation.

Though the study as to what happens to the metal during process of preparation of its Bhasma is of academic and research interest, it is essential to ensure that neither there is any toxicity due to the metal itself nor when it is added in herbomineral preparations. In fact as the metals are incorporated in such formulations with a view to perform their specific therapeutic role. And therefore to derive these benefits from such preparations, it is imperative that the formulations containing Bhasmas and metals prepared strictly as per the classical procedure, such

preparation when subjected to toxicity testing should ensure not only their efficacy but the safety as well.

### 1.3 SHWASKUTHAR RASA

The *Shwaskuthar Rasa* is a prestigious and potential herbomineral formulation of Ayurveda tested on hundred years of time scale for the treatment of Asthma, Allergy and other respiratory associated problems. The formulation comprises of four herbs - Vatsanabha (*Aconitum ferox*), Pippali (*Piper longum*), Maricha (*Piper nigrum*), Adarakha (*Zingiber officinale*) and four minerals - Parada (Mercury), Manahsila (Arsenic disulphide), Gandhaka (Sulphur) and Tankana (Borax) (**Acharya, 1979**). In Ayurveda *Aconitum ferox*, *Piper longum*, *Piper nigrum* and *Zingiber officinale* are the valued ingredients used in several of respiratory related formulations since time immemorial. Ayurveda also mentions the various aspects toxicity of these mercury and arsenic in detail. Therefore it is noticeable worth mentioning that in-spite of having a thorough knowledge of toxicity of these so-called heavy metal substances, they have been incorporated along with the herbal ingredients to prepare a herbomineral formulation by Ayurveda which was found much more effective compared to formulation prepared from herbal ingredients alone. However of late preparations like *Shwaskuthar Rasa* along with most Rasas and Bhasmas of Ayurveda have come under whirl-wind of a controversy by a report published in Journal of American Medical Association (JAMA), purely on the pretext that heavy metals present in these herbomineral formulation are toxic (**Robert et al., 2008**).

On a very straight forward statement made in JAMA, the Ayurvedic preparations containing so-called heavy metals have been declared unfit for human use. Such a conclusion seems to be immature and thus unfortunate as it lacks serious scientific thoughts without considering the whole herbomineral therapy of Ayurveda of high repute and long standing. It would be better and fitness in the things that before reaching

to any such conclusion, thorough research works with proper scientific approach is taken up considering the following aspects -

- a. The amount of heavy metals present in herbomineral formulation:** Herbomineral formulations usually contain relatively very less concentration of heavy metals that may be well below the toxicity limits.
- b. Small dose of herbomineral formulation is prescribed:** The relatively small doses of such preparations may further reduce the levels of so called heavy metals. As per literature herbomineral formulations are prescribed in only milligrams of doses.
- c. Prescribed for small duration:** As herbomineral preparations are highly potent and efficacious hence they are prescribed for shorter duration, thus further minimizing the amount of heavy metals in the organs / system / body.
- d. Preparations are prescribed mostly in acute conditions:** Being highly potent Rasas and Bhasmas of Ayurveda are prescribed to patients only in critical and acute conditions.
- e. Chemical status of heavy metals present in herbomineral formulation:** Heavy metals as such in metallic form may be toxic but it is also true that in salt form or in other complex forms heavy metals may be very less toxic or even non-toxic. Contrary to this, it is also possible that in such forms they may have improved therapeutic activity/value/bioavailability.
- f. Chemical status of heavy metals:** Before arriving to any such conclusion of discarding the age-old formulations on toxicity, it is rather essential to know / establish the chemical status of the metals that whether these metals are in divalent or trivalent form or in the form of some complexation with herbal constituents. It is very important to ascertain this fact as heavy metals in these forms or salts or as organometallic complexes may have different absorption, accumulation and therapeutic impact / toxicity compared to their pure status i.e. metal form. There lies another

challenge for researchers to look- into and explore-why mercury, arsenic, tin, lead, cadmium etc, were selected as therapeutic base metals for herbomineral preparations in ancient Indian medicinal system, besides lighter transition metal ions which are considered under life essential metal ions.

- g. Particle size prescribed for such formulation:** As per the method prescribed in Ayurveda, these herbomineral formulations are prepared by thorough trituration for a longer period extending sometime to weeks or even months thus rendering the particle size to a very small scale. In the light of present scientific scenario the particle size of final herbomineral formulation probably may reach to nano or near nano scale. Because of this small particle size, these formulations may be absorbed faster due to their increased surface area. Further the particles being very small may also cross through the different systemic barriers and after performing their therapeutic effect, by way of two way transport, the same may be excreted out through cell system with-out any bio-accumulation in the body. Thus a reduction in accumulation of so-called heavy metals in body organs may further decrease the chances of causing any toxicity in the body / body organs.
- h. Status of charge on particle of heavy metals:** As per prescribed procedure, in the final herbomineral formulation the metal should completely loose their metallic texture / nature. During preparation of Rasas and Bhasmas, the metal or metal salts are powdered or trituated in such a way and for such a long time that these are converted into very fine particles and they loose their metallic status or texture. In the whole process, it is quite possible that the metal particles might convert into ionic forms and may attain certain charges which may either potentiate the therapeutic value of combination or may help in targeting drug at desired site. It is also possible that specially the mercury might be acting as a vehicle to carry / transport the bio-actives to specific site. It is also

further possible that in such an ionic state, the so-called toxicity of heavy metals might be reduced considerably or may reach to even toxicity zero level.

- i. Complexation of heavy metals with other metals or phytopharmaceuticals:** During preparation of Rasas and Bhasmas of Ayurveda, the metals or metal salts are triturated thoroughly with herbal components, during which phytoconstituents present in herbs may interact / react with metal salts or metal ions forming the organometallics, the physical, chemical, physicochemical and therapeutic effects of which may be altered entirely.
- j. The purity of heavy metals required for preparing herbomineral formulations:** As per Ayurveda a very high level of purity of these metals, used for preparing formulations is required and for the purification of the metals, the Ayurveda prescribes very stringent procedures. It is quite possible that the impurity / impurities associated with these so-called heavy metals might be responsible for higher level of toxicity and the stringent purification procedure may reduce the toxic manifestation of heavy metals to a very great extent.
- k. Metal and mineral content of body:** Composition of human body are Oxygen (65%), Carbon (18%), Hydrogen (10%), Nitrogen (3%), Calcium (1.5%), Phosphorus (1.0%), Potassium (0.35%), Sulfur (0.25%), Sodium (0.15%), Magnesium (0.05%), Copper, Zinc, Selenium, Molybdenum, Fluorine, Chlorine, Iodine, Manganese, Cobalt, Iron (0.70%) and trace elements are Lithium, Strontium, Aluminum, Silicon, Lead, Vanadium, mercury, Arsenic and Bromine in negligible amount (**Harper et al., 1977**). Nearly all metals are present in the earth's crust which enters the human bodies continuously at low levels through the food. It is a common mistake, based on fear and misinformation, to believe that a toxin has a linear toxic effect down to the lowest levels. All toxins have a safe threshold below which there is no toxicity. In fact, below a safe

threshold toxicity disappears and there is no toxicity at all and in some cases even beneficial exists. Mercury is most widely used metal in Rasa-Shastra discipline of Ayurveda and to some extent it is most controversial also. Recent developments have highlighted the need to research of its use.

1. **WHO stand on so-called heavy metals:** As per the World Health organization (**WHO, 2004b**) also the so-called heavy metals are safe for human consumption to certain limits which are 10 ppm for lead, 1ppm for mercury, 0.3 ppm for cadmium and 10 ppm for arsenic. Thus a thorough study on all these aspect is vital before arriving to any conclusion of condemning the highly reputed age old herbomineral formulations for human use.

**Shwaskuthar Rasa dose:** 125 to 250 mg per day, oral (**AFI, 2000**)

**Therapeutic use:** Agnimandya, kasa, svasa roga, vatakaphaja roga, sannipata roga, apasmara and meha etc.

#### 1.4 SHODHANA OF METALS

Considering therapeutic value the use of metals minerals and gems etc in Ayurvedic preparation is common but due to ignorance and improper information some scientists arising question about the rationality of using these metals for therapeutic purposes. Actually the metals available in nature are either in combination with undesired other elements or in improper and non consumable forms. These forms of metals are not suitable for human body. All modifications and developments including shodhana process in Rasa Shastra are for the purpose of making these elements useful and body friendly (**Shastry, 1999**).

Shodhana is a process of removal of impurities from substances by using different processes of swedana, mardana etc with particular drugs. It is a process by which blemishes are separated from the substance by various processes like grinding etc with specific drugs.

The two basic processes adopted for this purpose are shodhana and marana. Shodhana is the preliminary but most important procedure of Ayurveda to make metals free from toxicity, potentiate them to achieve the therapeutic excellence and to make them easily digestible, absorbable and assimilable.

Shodhana process described in Rasa texts is not only a process of chemical purification but it is a specific process of addition and separation which causes physical, chemical and biological changes in the metals (**Sharma, 1981; Tripathi, 1995**). These changes depend on the structure, constituents, impurity and properties of particular substance.

#### **1.4.1 Types of shodhana**

Literally shodhana means detoxification, though references regarding shodhana are available since the times of Charaka Samhita (1000 B.C. to 500 B.C.). The details about this treatment could be traced only after the development of Rasa Shastra i.e. from 8<sup>th</sup> century AD and onwards. During that time number of processes were developed for purifying the mineral / metal drug to remove their toxicity. As these drugs have many desirable qualities, the only disadvantage is that they have high toxicity and little absorption capacity (**Mitra et al., 1999**). So after considering this view Acharya's developed different shodhana treatments for different types of drugs depending on their different physical and chemical characteristics to make them suitable and useful for the body.

##### **1.4.1.1 Samanya shodhana**

This is used as a general procedure for shodhana of all drugs of a particular group. e.g. Metals

##### **1.4.1.2 Vishesha shodhana**

It is used as a specific procedure for a particular drug material. It can be applied after samanya shodhana e.g. Swarna shodhana with panchamritika.

**Table 1.2 Different methods of shodhana**

S.No	Shodhana	Process	Example
1	Swedana	Boiling with liquid	Sankha shodhana
2	Mardana	Trituration	Parada shodhana
3	Murchana	Trituration up to fine disintegration	Parada shodhana
4	Patana	Sublimation	Parada shodhana
5	Abhiseka	Sprinkling	Mandura shodhana
6	Atapa	Drying	Lauha shodhana
7	Acushana	Absorption	Bhallataka shodhana
8	Bhavana	Levigation	Hingula shodhana
9	Bharjana	Frying	Hingu shodhana
10	Dhalana	Melting and quenching	Vanga shodhana
11	Galana	Melting and pouring	Gandhaka shodhana.
12	Nirjalikarana	Evaporation of water	Sphatika shodhana
13	Nirvapa	Heating and Quenching	Samanya shodhana of metals
14	Prithakikarana	Separation	Gugglu shodhana
15	Vilayana	Elutriations	Silajitu shodhana
16	Prakshalana	Washing	Godanti shodhana

**1.4.2 Process of shodhana**

- a) Heating and dipping:** It is a common method applicable specially for metals, gems, minerals etc. This method of purification is applicable for the drugs/minerals which are very hard in nature as copper, mica, mandura, diamond etc.
- b) Heating, melting and pouring into liquids:** This method is indicated for the drugs having low melting point e.g. Zinc, sulphur etc.
- c) Boiling with liquids:** It is a common method of purification called as swedana. This method is applicable when drug is having impurities soluble only in hot acidic, alkaline or oily media e.g Mercury, manahsila, hartala and drugs of sudha varga etc.

- d) Distillation:** This method is applied when drug is having low vaporization point e.g., Mercury, sulphur and ammonia salt etc.
- e) Trituration with herbal drugs (bhavana):** The process is applied to drugs that are soft in nature. Actually this process exposes the surface area of drug and each particle comes into direct contact with purifying drug/substance e.g. Mercury, arsenic etc.
- f) Soaking in liquids:** Mainly applicable in case of vegetable drugs and poisons e.g. Aconite and nuxvomica etc
- g) Frying:** This method is applied when drugs either contain water of crystallization or volatile impurities e.g Tankana (borax), sphatika (alum) and hingu (asafoetida) etc.

### **1.4.3 Importance of shodhana**

#### **1.4.3.1 Physical changes during shodhana**

- Elimination of physical impurities
- Reduction in hardness
- To increase brittleness – due to repeated heating and quenching micro cracks are developed on the surface of metals.
- Reduction in particle size

#### **1.4.3.2 Chemical changes during shodhana**

- Elimination of chemical impurities
- Formation of compounds e.g. during red hot stage oxidation occurs in metals and non-metals and oxides are formed.

#### **1.4.3.3 Biological changes during shodhana**

The physicochemical changes ultimately increase bioavailability and reduction in particle size helps in absorption due to increased surface area. Smoothness leads to non-irritability and chemical changes make the metals body friendly and suitable for ingestion.

### **1.4.4 Basic structure of metals**

Metals are solid crystalline in nature. The normal metallic object consist of an aggregate of many small crystals and thus metal are polycrystalline. The crystals in metals normally called as grains are



- When temperature is applied, the particles gain energy and vibrate strongly and displacement of equilibrium occurs, intra-atomic distance increases and solid gets expanded.
- Due to increase in intra-atomic distance, electrostatic forces get weakened.
- Due to continuous heating particles get enough energy to break forces and thus can move around. This expansion is called linear expansion.
- During red hot condition metals react with atmospheric oxygen and compounds are formed on the surface. Generally expansibility of compounds are less than metals which leads to separation of compound from metal.

**Phase of Quenching:** After heating and immediate cooling in liquid media leads to decrease in tension and increase in compression force. The media immediately penetrates inside and media soluble impurities get dissolved. Repetition of heating and cooling causes disruption in equilibrium, leads to increased brittleness, reduction in hardness and finally reduction in particle size.

**Post Quenching Interaction:** After nirvapa and during instant cooling re-crystallization occurs along with reformation of grain boundaries. Each grain is surrounded by the molecules of liquid media imposing its properties on the purified metals.

#### **1.4.6 Role of media in samanya shodhana of metals**

Media plays an important role in shodhana process and that is the reason of using specific media for shodhana of a particular substance. Media can act in different ways -

- Sometimes media acts as solvent to dissolve or soluble impurities.
- Sometimes media acts to eradicate toxic substance from the drug.
- Media provides some organic and inorganic principles to the material which have important role in the body.
- Sometimes media helps in physical transformation of some metals and minerals.

- Fate of metal deformation depends on the nature of liquid used for quenching

According to Ayurveda, In samanya shodhana of metals, materials taken for shodhana are heated red hot and dipped into various cold liquids i.e. Tail, takra, gomutra respectively for 7 times in each liquid. The order of these media for shodhana is important. Intensity of tikshnatva increases in rising order in case of these media from tail up to gomitra. These media nullify mala (excretion), kathinata (pain in abdomen), jadta etc. The media used for samanya shodhana process have one or more properties as below.

- Weak / Strong acids
- Weak / Strong bases
- Enzymes
- Solvent property
- Inorganic content
- Organic content

## 1.5 NANOTECHNOLOGY

Nanotechnology is a field of applied science and technology covering a broad range of topics and cutting across many disciplines, including colloidal science, chemistry, applied physics and biology.

Two main approaches are used in nanotechnology, one is a "bottom-up" approach which is simple to complex approach where materials and devices are built from molecular components which assemble themselves chemically using principles of molecular recognition; the other being a "top-down" approach i.e. larger to smaller where nano-objects are constructed from larger entities without atomic-level control.

### 1.5.1 Bottom-up approaches (Simple to complex)

These approaches utilize the concepts of molecular self-assembly and/or supramolecular chemistry to automatically arrange themselves into some useful conformation through a "bottom-up" approach. The concept of molecular recognition is especially important where molecules

can be designed so that a specific conformation or arrangement is favored due to non-covalent intermolecular forces. The process arranges smaller components into more complex assemblies.

- DNA nanotechnology utilizes the specificity of Watson-Crick base pairing to construct well-defined structures out of DNA and other nucleic acids.
- More commonly, molecular self-assembly seeks to use concepts of supramolecular chemistry, and molecular recognition in particular, to cause single-molecule components to automatically arrange themselves into some useful conformation.

### **1.5.2 Top-down approach (Larger to smaller)**

The electronic properties of solids are altered greatly with reductions in their particle size. It has been observed that materials reduced to the nanoscale suddenly show very different properties compared to what they exhibit on a macroscale, enabling their unique applications. Another concern is that the volume of an object decreases as the third power of its linear dimensions ( $V \propto r^3$ ), whereas the surface area only decreases as its second power ( $S \propto r^2$ ). This somewhat subtle and unavoidable principle has huge ramifications.

### **1.5.3 Molecular nanotechnology: A long-term view**

Advanced nanotechnology, also called molecular manufacturing, is the concept of engineered nanosystems operating on the molecular scale. By the countless examples found in biology it is currently known that billions of years of evolutionary feed back can produce sophisticated, optimized biological machines.

### **1.5.4 Nanotechnology vis-a-vis body system**

Although ill effects of nanomaterials are possible, they are not well studied yet. However there are efforts now to investigate the effects of nanomaterials on animals. Particle size around 50 nm can affect the cells and 30 nm can affect central nervous system. Endings of our respiratory track consist of small packets known as alveoli, which are 0.3 $\mu$ m in size.

They get affected by particles of size around 70 nm. In general small particles with size  $<0.1\mu\text{m}$  can affect our respiratory system and other organs. However details about nanomaterials and size dependent effects are yet to be known.

### **1.5.5 Effect of particle size on absorption and bioavailability of drugs**

Particle size and surface area influence the release of drug from dosage form that is administered. Higher surface area brings about intimate contact of the drug with the dissolution fluids *in vivo* and increases the drug solubility and its dissolution. In general, higher the surface area, better the release and hence faster the dissolution.

Particle size and surface area influence the drug absorption and subsequently the therapeutic action. Thus higher the dissolution the faster will be the absorption and action of drug.

### **1.5.6 Factors affecting the size reduction**

**Hardness:** Hardness of the material is a surface property and carries a meaning different from what is referred to as strength. A very hard but brittle material should present no special problem in size reduction.

**Toughness:** A soft but tough material may present more problems in size reduction than a hard but brittle substance.

**Physical nature:** Friable materials like sucrose and dried filter cake tend to fracture along well defined plains and may be comminuted by attrition, impact or pressure where as fibrous materials like glycyrrhiza and rauwolfia cannot be crushed by pressure or impact and must be subjected to cutting action.

**Moisture content:** The presence of more than 5% water content generally hinders the size reduction and therefore less than 5% moisture is suitable for dry grinding.

Other product specification such as size and shape of the milled particle, ratio of the feed to product size, desired flow properties and bulk density also influence the choice of grinding/pulverizing equipments.

### 1.5.7 Methods for size reduction

Size reduction involves following mechanisms.

**Cutting:** It implies hitting of more or less stationary materials by means of sharp blade.

**Compression:** It is accomplished by application of force by a suitable device.

**Impact:** Impact implies hitting of a more or less stationary material by an object moving at high speed or striking the moving particles at a stationary surface.

**Attrition:** Attrition occurs when the material is subjected to pressure as in compression but the surfaces are mobile in relation to each other, resulting in shear forces which break the particle.

**Trituration:** It is the circular motion causes blending which breaks up soft aggregates of powders.

**Pulverization by intervention:** This is the process of powdering a substance with the help of other substance which can be removed easily after the pulverization has been completed.

**Levigation:** The reduction of a substance to an extremely fine state of subdivision by rubbing it in a slab with the aid of an insoluble liquid called levigation.

### 1.5.8 Size reduction equipments

Size reduction can be achieved through following equipments.

Hand mill	Disintegrator
Laboratory mill	Chaser mill
Cutter mill	Ball mill
Roller mill	Fluid energy mill
Hammer mill	Colloid mill

### 1.5.9 Nanomaterials or nanoscience

Nanomaterial is a field of study of materials having unique properties arising from their nanoscale dimension or nano sized form.

- Colloid science has given rise to many materials which may be useful in nanotechnology, such as carbon nanotubes, fullerenes, various nanoparticles and nanorods.
- Headway has been made in using these materials for medical applications; e.g. Nanomedicine

### 1.5.10 Nanoparticles

Nanoparticle is an amorphous or semicrystalline nano-structure having diameter less than  $1\mu\text{m}$ . Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale, this is often not the case. The properties of materials change as their size approaches to near nano or nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometre the percentage of atoms at the surface is minuscule relative to the total number of atoms of the material. The interesting and sometimes unexpected properties of nanoparticles are partly due to the aspects of the surface of the material dominating the properties in lieu of their bulk properties (**Fahlman, 2007**).

Nanoparticles exhibit a number of special properties relative to bulk material eg. bending of bulk copper (wire, ribbon, etc.) occurs with movement of copper atoms / clusters at about the 50 nm scale. Suspensions of nanoparticles are possible because the interaction of the particle surface with the solvent is strong enough to overcome differences in density, which usually result in a material either sinking or floating in a liquid. Nanoparticles also often have unexpected visible properties because they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep red to black in solution (**Moyses et al., 2010**).

Nanoparticles have a very high surface area to volume ratio. This provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over

shorter time scales than for larger particles. This theoretically does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. The large surface area to volume ratio also reduces the incipient melting temperature of nanoparticles (**Fahlman, 2007**).

#### **1.5.11 Fabrication of nanoparticles**

There are several ways for creating nanoparticles and attrition and pyrolysis are common methods. In attrition, macro or micro scale particles are ground in a ball mill, a planetary ball mill, or other size reduction device. In pyrolysis, an organic precursor (liquid or gas) is forced through an orifice at high pressure and burnt.

#### **1.5.12 Nanotechnology as applied to biological systems**

**Size:** An advantage of nanotechnology as it relates to biological systems is its ability to control the size of the resulting particles and the devices. Nanoscale devices and components are of the same basic size as biological entities whereas nanoscale constructs are smaller than human cells (10-20 thousand nanometer in diameter) and organelles and similar in size to large biological macromolecules such enzymes, adrenoreceptors and haemoglobin, which is nearly 5 nm in diameter, and the lipid bilayer surrounding cells is of the order of 6 nm thick. Nanoparticles, smaller than 20 nm, can transit through walls of blood vessels. Nanoparticles also offer the ability to penetrate the blood-brain barrier or the stomach epithelial barriers which make it difficult for large therapeutic agents to reach their intended targets. To be suitable as a drug-delivery platform, the size of nanoparticles must be small enough to avoid rapid filtration by the spleen, with filaments spaced at roughly 200 nm, which serve as a mesh work for phagocytotic cells. Similarly, to traverse the liver, the particles must be small enough to pass through the organ's 150–200 nm size and avoid the Kupffer cell-lined sieve plates.

**Solubility:** Nanoparticles with hydrophilic polymers such as PEG attached to their surface can act as a platform for lipophilic molecules and overcome the solubility barrier. Insoluble compounds can be

attached, adsorbed or otherwise encapsulated in the hydrated nanoparticles. Solubility of the composite entity subsequently becomes a function of the nanoparticle carrier rather than being strictly dependent on the drug itself.

### 1.5.13 Testing of nanoparticles

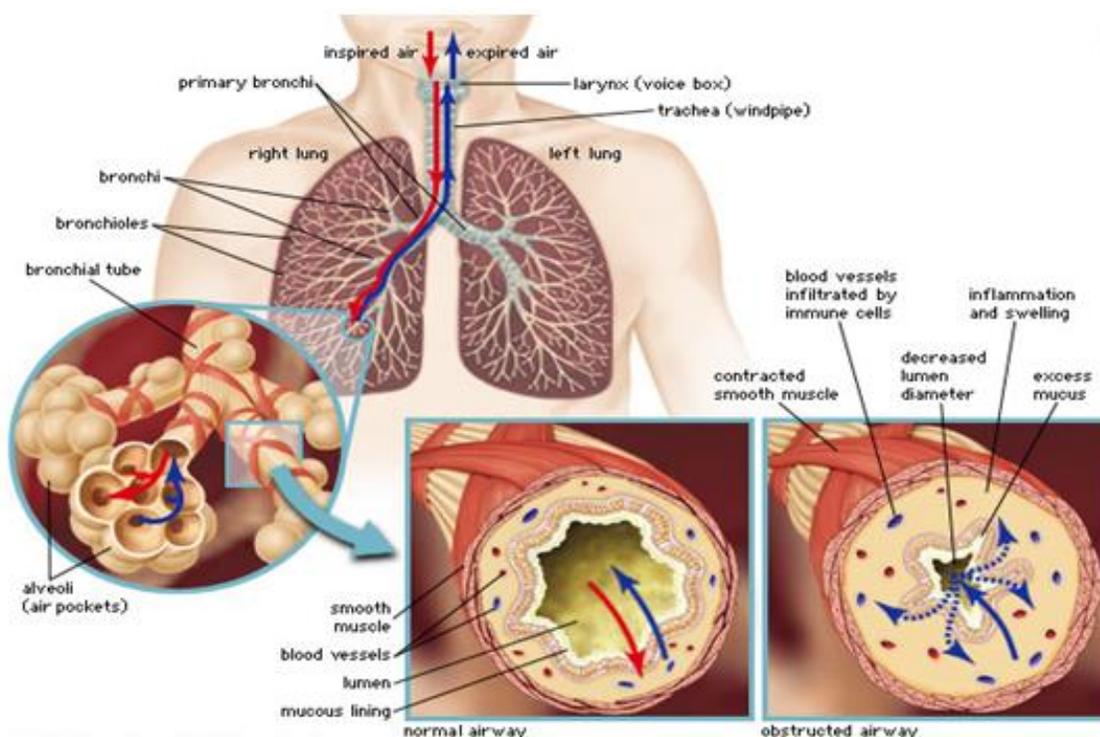
The impact of particle size of the formulation was known to Ayurvedic practitioners of olden times. Accordingly to assess the desired particle size they prescribed stringent testing procedure for certain formulation form including Rasas and Bhasmas. The process of nanoparticle testing in Ayurvedic products involves following basis.

- First, one needs to establish the presence of nanoparticles in the test sample.
- Subsequently it is necessary to ascertain whether the composition is homogeneous.
- The third test is on the state/status of the nanoparticles, as to whether they are crystalline or amorphous; and the nature of defects in the sample.
- Followed to it the sample has to be biologically tested to check its bio-activity.
- Finally, the convergence of all these factors in the mechanism of action for a particular application needs to be tested.

## 1.6 ASTHMA

Asthma is a commonly occurring condition that is most difficult to control in chronic stage. In the United States alone, asthma affects almost 17 million people, and there has been a 75% increase in asthma cases in the last 20 years meaning thereby that about 1 out of every 20 adults and close to 1 out of 13 children today have asthma or related problem. In school age children, asthma has risen by 75%. India alone has an estimated 15-20 million asthmatics. Mortality data from developed countries show that the rate varies from 0.1- 0.8 per lac persons aged 5-34 years (**Nichols and Longsworth, 2000**).

Asthma is a disease of airways characterized by chronic airway inflammation (**Eunhyung et al., 2006**) and airway hypersensitivity to a variety of stimuli and airway obstruction (**Djukanovic et al., 1990**). Airway obstruction due to smooth muscle spasms in the walls of smaller bronchi and bronchioles and edema of the mucosa of the airways, increases mucus secretion and damages the epithelium of airway (**Gerard and Derrickson, 2006**). Increased numbers of inflammatory cells including eosinophils, basophils, macrophages and lymphocytes can be found in bronchoalveolar lavage fluid from asthmatic patients (**Joel and Lee, 2001**).



**Photograph 1.1 Anatomy of asthma**

### 1.6.1 Types of asthma

Based on the stimuli initiating bronchial asthma, two broad etiologic types are traditionally described; extrinsic asthma and intrinsic asthma. A third type is a mixed pattern in which the features do not fit into either of the two main types (**Clark and Hageman, 2007**).

### 1.6.1.1 Extrinsic asthma (Atopic, allergic)

The most common type of asthma usually begins in childhood or in early adult life (**Burr, 1999**). Most patients of this type of asthma have personal or family history of preceding allergic diseases such as rhinitis, urticaria or infinite eczema. Hypersensitivity to various extrinsic antigenic substance or allergens is usually present in these cases. Most of the allergens cause ill-effects by inhalation e.g. house-dust, pollens, animal danders and moulds etc. Occupational asthma stimulated by fumes, gases, organic and chemical dusts is a variant of extrinsic asthma. There are increased levels of IgE in the serum and positive skin test with the specific offending inhaled antigen representing an IgE mediated type-I hypersensitivity reaction which includes an 'acute immediate response' and 'late phase reaction'.

**Acute immediate response:** It is initiated by IgE sensitized mast cells on the mucosal surface. Mast cells on degranulation release histamine, leukotriens, prostaglandins, platelet activating factor and chemotactic factors for eosinophils and neutrophils causing broncho-constriction, oedema, mucus hypersecretion and accumulation of oesinophils and neutrophils.

**Late phase reaction:** The acute immediate response is responsible for the prolonged manifestations of asthma (**Rang and Dale, 2003**). It is caused by excessive mobilization of blood leucocytes including basophils besides eosinophils and neutrophils. These result in further release of mediators accentuating the above mentioned effects. In addition, inflammatory injury is caused by neutrophils and by major basic protein of eosinophil.

### 1.6.1.2 Intrinsic asthma (Idiosyncratic, non-atopic)

It is developed in later adult life with family history of allergy, negative skin test and normal serum levels of IgE. Most of the patients develop typical complex symptoms after an upper respiratory tract infection by viruses. Associated nasal polypi and chronic bronchitis are commonly present. Although there are no recognizable allergens but

about 10% of patients become hypersensitive to drugs, most notably to small doses of aspirin (**Harsh Mohan, 2006**).

### 1.6.1.3 Mixed type asthma

Many patients do not clearly fit into either of the above two categories and have mixed features of both. Those patients who develop asthma in early life have strong allergic components, while those who develop the disease late tend to be non-allergic. Either type of asthma can be precipitated by cold, exercise and emotional stress.

**Table 1.3 Contrasting features of major types of asthma**

Feature	Extrinsic asthma	Intrinsic asthma
Age at onset	Childhood	Adult
Personal family history	Commonly present	Absent
Preceding allergic illness (rhinitis, eczema)	Present	Absent
Allergens (dust, pollens)	Present	None
Drug Hypersensitivity	None	Present
Serum IgE levels	Elevated	Normal
Associated chronic bronchitis, nasal polyps	Absent	Present
Emphysema	Unusual	Common

### 1.6.2 Etiology

Triggering factors for bronchial asthma are allergens, irritants, exercise, infection, drugs, exposure to cold air and psychological stimuli release of histamine, bradykinin, serotonin, prostaglandins, leukotriens, platelet activating factors, tumor necrosing factors (TNF- $\alpha$ ), interleukin and extracellular fluid etc that leads to smooth muscle contraction, muscle congestion and exudation (**Barar, 2007**).

### 1.6.3 Signs and Symptoms

Symptoms of asthma are coughing, wheezing, dyspnoea, chest tightness, tachycardia, fatigue, moist skin and anxiety.

#### 1.6.4 Diagnosis and test for asthma

The diagnosis of asthma depends on a detailed and enlightened evaluation of history and other tests, measurement and appropriate follow up. The important laboratory tests useful in diagnosis of asthma include -

- A. **Skin test:** To detect specific IgE antibody – A positive immediate skin test reaction is a function of IgE antibody for a specific allergen, the release of mast cell mediators and the reactivity of patient's skin to histamine (**Berkaw, 1992**).
- B. **Eosinophilia:** An abnormal increase in peripheral eosinophils to more than 4 % total leukocytes count is termed as eosinophilia (**Brigden, 1999**). In asthma patients, there is an increase in eosinophil count.
- C. **Sputum:** It is highly distinctive, normally it is tenacious, whitish and rubbery. But in case of infection and asthma it is yellowish.
- D. **Pulmonary function test:** These test are valuable in diagnosis of different disorders and also in known asthmatics in order to assess the degree of airways obstruction and disturbance in gas exchange. To measure the airways response to inhaled allergens and chemicals, to quantify the response to drugs and for long term follow up. Pulmonary function test are more valuable when performed before and after giving aerosolized bronchodilator to determine the degree of reversibility of the airways obstruction.
- E. **Chest X-ray:** In asthma patients the marking on lungs are increased particularly in chronic disease. Inspiratory and expiratory chest X-ray helps to diagnose body expiration as a cause of wheezing. Leakage of cells in tracheobronchial airways can also be observed.

#### 1.6.5 Inflammatory cells and mediators of asthma

At the cellular and molecular level, the disease is currently described by a complex sequence of events that have been divided into the immediate or early responses and delayed responses. The immediate

response is mast cell mediated and results in rapid broncho-constriction within 10-20 min of allergen exposure and resolves within 1-2 hrs.

Delayed or late response is characterized by the involvement of eosinophils, lymphocytes, neutrophils and alveolar macrophages. Eosinophils have been identified as playing a central role in the pathogenesis of the late asthmatics response (**DeMonchy et al., 1985**).

### **1.6.6 Modulation of cells implicated in asthma**

The cellular involvement in asthma and related allergic conditions is a critical component of the pathogenesis of asthma. Evidences to support this concept include identification of various inflammatory cells types in asthmatic lung, the capacity of these cells to synthesize and release allergic mediators and the ability of these factors to induce various facts of the asthmatic response.

#### **1.6.6.1 Mast cells**

Ever since the identification of mast cells by Paul Ehrlich nearly 100 years ago, the mast cells has been intensively studied by various investigators. Mast cells are large round cells (15 to 20  $\mu\text{m}$  in diameter) distributed throughout the body in connective tissues. The presence of large amounts of histamine in mast cells (**Riley et al., 1953**) and the ability to synthesize other bioactive mediators in response to appropriate stimuli have placed the mast cells in a central role in the pathogenesis of asthma. Mast cells are found in the connective tissues of the lung and are often situated close to microvasculature, lymphatics, bronchial lumen, submucous glands and throughout smooth muscle bundles (**Galli et al., 1984**). They do not circulate and thus the consequence of mast cell activation depends largely on existing distribution. Mast cells participate in IgE-mediated reactions and cross linking of immunoglobulin IgE receptors cause the release of the biologically active mediators. Although basophils share several notable properties with mast cells such as being able to bind IgE and release histamine (**Conroy et al., 1977; Schulman et al., 1988**).

### 1.6.6.2 Macrophages

Macrophages are abundant throughout the respiratory tract and recent evidence that they may be activated by IgE-greater dependent mechanism has suggested their involvement in allergic inflammation (**Joseph et al., 1983**). Macrophages from asthmatic patients release greater amounts of mediators such as thromboxane, prostaglandin and platelet activating factors (PAF) compared to those derived from normal subjects. Interestingly, human lung macrophages are potentially inhibited by corticosteroids (**Fuller et al., 1984**).

### 1.6.6.3 Eosinophils

Eosinophil infiltration is a prominent feature of asthma and differentiates asthma from other inflammatory conditions of the airway. Antigen inhalation results in a marked increase in eosinophils in bronchioalveolar lavage at the time of the late reactions (**DeMonchy et al., 1985**) and there is relationship between peripheral blood eosinophilia and bronchial hyper-responsiveness. Eosinophils may release a variety of mediators, including leukotrienes and platelet activating factor (**Lee et al., 1984**) and also proteins such as major basic protein and eosinophil cationic protein which are toxic to airway epithelium.

### 1.6.6.4 Neutrophils

Neutrophils are also found in asthmatic airways and may also release a number of mediators including Leukotriene B<sub>4</sub> (LT B<sub>4</sub>), prostaglandins, PAF and adenosine. In animal models of bronchial hyper-responsiveness, neutrophils have been implicated (**Murphy et al., 1980**) but their role in asthmatic airways remains less defined than that of eosinophils.

### 1.6.6.5 Epithelial cells

Airways epithelial damage is a common feature of even mild asthma and this may underlie bronchial hyper responsiveness (**Laitinen et al., 1985**), since many of the conditions known to increase bronchial responsiveness (ozone exposure, upper respiratory tract viral infection,

allergen exposure) are associated with epithelial damage. Epithelial cells may themselves also release inflammatory mediators such as Leukotriene B<sub>4</sub> (LT B<sub>4</sub>) and 15-hydroxy-5, 8, 11, 13-eicosetraenoic acid (15 HETE) which are chemotactic for inflammatory cells.

#### **1.6.6.6 Platelets**

Abnormalities in platelet functions have been found in asthma and animal studies suggest that platelets are involved in bronchial responsiveness (**Court et al., 1987**). Platelets may release a variety of mediators such as serotonin, thromboxane 5 and 12-lipoxygenase products, PAF and oxygen free radicals and may be activated by IgE-dependent mechanisms.

#### **1.6.6.7 Cellular origin of mediators in asthma**

Various inflammatory cells release a heterogeneous group of mediators which differ in their potency and biological activities. These mediators are both pleiotropic and redundant; i.e. each mediator has more than one function and mediators may overlap in their biological effects. For instance, histamine alters vasopermeability and induces mucous secretion, the properties it shares with leukotriene C<sub>4</sub>.

Mast cell granules contain high concentrations of histamine and (in some species) serotonin. Although these two compounds are important mediators of inflammation, they are only part of the total spectrum of inflammatory mediators released following mast cell degranulation. More than half the preformed mast cell granules consist of trypsin or chymotrypsin like neutral proteases. These proteases can destroy nearby cells and activate complement components C<sub>3</sub> and C<sub>5</sub> to generate anaphylatoxins.

Platelet activating factor (PAF) is a mixture of phospholipids closely related to lecithins. It is synthesized by mast cells following exposure to antigen or anaphylatoxins and by platelets. PAF makes platelet aggregate and release their vasoactive molecules, especially serotonin and synthesize thromboxanes. PAF promotes neutrophil aggregation, degranulation, chemotoxins, the release of oxygen radicals and a

neutropenia, although their precise effect does depend on the molecular species of PAF involved.

As allergies or Type-I hypersensitivities are inflammatory reactions mediated mainly by IgE bound to mast cells and basophils and these reactions result from the release of pharmacologically active mediators from these cells.

### **1.6.7 Preformed chemical mediators**

#### **1.6.7.1 Biogenic amines**

Biogenic amines have been detected in mast cells of mammals, avian and reptiles except in those of fish and amphibians. Histamine is the major biogenic amine in human mast cells and basophils and in rodent mast cells. Serotonin a monoamine is present in rodent connective tissue mast cells (**Weitzman et al., 1985**) but not in rat intestinal mast cells, while dopamine is present in bovine mast cells.

**Histamine:** It is formed in mast cells and basophils from histidine (**Laguneff, 1981**) by histidine decarboxylase and is then stored in the secretory granules where at the acidic pH of the unstimulated cell, it is associated by ionic linkage with carboxyl groups of proteoglycans and proteins. Histamine is the only preformed mediator of human mast cells with direct potent vasoactive and smooth muscle spasmogen. Rat peritoneal mast cells contain 10-30 pg histamine/cell. Histamine is dissociated from the proteoglycans protein complex by cation exchange with extra-cellular sodium at neutral pH. Specific receptors present on target cell surfaces then mediate the wide ranging biological activities of histamine.

Histamine exerts its biological and pathobiological effects through its interaction with cell specific receptors designated H<sub>1</sub>, H<sub>2</sub>, and H<sub>3</sub>. The interaction of histamine with H<sub>1</sub> receptors result in the contraction of airway and gastrointestinal smooth muscle and vasospasm. The distinction between H<sub>1</sub> and H<sub>2</sub> receptors is based on the inhibitory activity of certain drugs on specific pharmacological effects of histamine. Effects of histamine mediated by H<sub>1</sub>, receptors include enhanced

vasopermeability between venular endothelial cells, vasodilation, contraction of bronchial and gastrointestinal smooth muscle and increased mucus secretion at various sites. The combined effects of H<sub>1</sub> and H<sub>2</sub> receptor mediated activities of histamine are required for the full expression of vasoactivity. For example the triple response caused by an intradermal injection of histamine namely central erythema (histamine arteriolar vasodilation), circumferential erythema (axon reflex vasodilation) and a central wheal (histamine vasopermeability, oedema) is most blocked by H<sub>1</sub> receptor or by H<sub>2</sub> receptor antagonist but is completely blocked only with a combination of H<sub>1</sub> and H<sub>2</sub> receptor antagonist.

**Bradykinin:** It is a neuropeptide released during the inflammatory response is known to play an important role in allergic pathophysiology of the airways, contributing to bronchoconstriction and edema formation. Raised levels of kinin generating enzymes and kinin are found in the airways during allergic response (**Farmer et al., 1991**). In asthmatic patient, inhaled bradykinin is one of the most potent bronchoconstriction agents which stimulate the release of inflammatory mediators such as PAF, LTB<sub>4</sub> as well as various prostoglandins in many tissues including those of the airways.

**Platelet activating factor:** It has been detected after activation of mouse bone marrow derived mast cell, whereas production of PAF without its secretion has been observed with preparation of human mast cells (**Schleimer et al., 1986**).

#### **1.6.7.2 Proteoglycans**

The proteoglycans heparin and chondrotin sulfate E have been associated with human mast cells. Both heparin and chondrotin sulfate E stabilize mast cell protease and alter the biological activity of many enzymes. Heparin is also a potent anticoagulant. The presence of highly sulphated proteoglycans in secretory granules of mast cells and basophils results in metachromasia when these cells are stained with basic dyes. The biological functions of endogenous mast cell

proteoglycans are somewhat speculative. These proteoglycans bind to histamine, neutral protease and acid hydrolase at the acidic pH inside mast cell secretory granules and may facilitate uptake and packaging of these preformed mediators into the secretory granules. Mast cells in different species and at different stages of development express varied combinations of granule proteases, i.e the “mast cell granule protease phenotype”. Rat mast cell protease or chymase is a neutral protease detected in mast cells of skin, muscle, parenchyma, pleural and peritoneal cavities of rat. Rat mast cell protease-II is chemically and antigenetically distinct and is located to mucosal mast cells of the gastrointestinal and respiratory tract.

Human mast cells contain the same three classes of proteases observed in mouse mast cells. Tryptase is the predominant enzyme associated with all human mast cells. It is stored in a manner similar to that of rat chymase, bind to heparin and is released as a complex with the proteoglycan.

#### **1.6.8 Newly generated mediator metabolites of arachidonic acid**

Activation of mast cells not causes the release of preformed granule associated mediators and initiates the de-nova synthesis of lipid derived substances. Of particular importance are the cyclo-oxygenase and lipo-oxygenase metabolites of arachidonic acid because these products possess potent inflammatory activity. Cyclo-oxygenase products include prostaglandin and thromboxanes, whereas lipo-oxygenase generates leukotrienes (LTs), hydroperoxyeicosatetra-aenoic acids (HPETES) and the reduced products of the HPETES.

Prostaglandin (PG) D<sub>2</sub> is generated after immunological activation by dispersed and purified human mast cells derived from lung, skin and bowel. PGD<sub>2</sub> levels in biological fluids have been used as a criterion to evaluate the differential involvement of mast cells and basophils in allergic reactions.

Leukotrienes derived from lipo-oxygenase pathway, enhance vascular permeability, constrict rat arteriolar (**Pfeffer et al., 1983**),

intestinal and bronchial smooth muscles and enhance bronchial mucus secretion. An important role for these molecules in asthma has been postulated based upon their potent bronchospastic activities, resistance to inhibition to available drugs secretion *in vitro* from allergen challenged lung tissue obtained from asthmatics and their mucosecretory activity.

### 1.6.9 Allopathic treatment for asthma

According to National Heart Lung and Blood Institute and Global Initiative for Asthma Medication the treatments for asthma can be divided into:

#### Quick relief medication: For emergency

- A. **Short acting adrenergic  $\beta_2$  agonists** eg. Albuterol, Bitolterol, Fenoterol, Isoetharine, Metaproterenol, Pirbuterol, Salutamol and Terbutaline etc
- B. **Anticholinergics** eg. Ipratropium bromide, Axitropium bromide, and Telenzepine etc
- C. **Short acting methylxanthines** eg. Aminophylline, Adrenaline injection and Theophylline etc

#### Long term preventive medication

- A. **Corticosteroids** eg. Adrenocorticosteroids, Glucosteroids, Inhalers - Beclomethasone, Budesonite, Flunisolide, Fluticasne and Triamcinolone  
Tablets and syrups – Methyl prednisolone, Prednisolone
- B. **Long acting  $\beta_2$  agonists**  
Inhalers – Farmoterol, Salmeterol  
Sustained released tablets – Salbutamol, Terbutaline
- C. **Antileucotrienes** eg. Montelukast, Zafirlucast and Zileuton
- D. **Mast cell stabilizers** eg. Sodium cromoglycate, Ketotifen
- F. **Other sustained release preparations** eg. Aminophylline and Methylxanthine

**1.6.10 Ayurvedic treatment for asthma****1.6.10.1 Shodhan chikitsa (Elimination)**

**Snehapan (Cleaning):** In this mode certain medicated *ghee* or oils are advised for ingestion. Usually *ghee* like *Vasa ghruta*, *Kantkari ghruta*, *Bharngyadi ghruta*, *Yashtimadhu ghruta* etc are used for shodhan purpose. These are administered in an increasing dosage schedule for not more than 7 days.

**Swedan (Sweating):** In acute stage, lukewarm *Mahanarayan taila* mixed with *saindhav* salt (rock salt) is used for gentle chest massage is followed by formentation by vapours of *dashmoola* decoction. It is a very effective remedy for reliving bronchospasm. Swedan by means of dry *valuka pottali* (*sand bag*) and hot water bag is also useful in acute cases.

**Vaman (Vomiting):** It reduces the recurrence rates of asthmatic attacks

**Virechan (Purgation):** It is an useful mode of purification for increasing the immunity of an individual towards allergies.

**Basti (Enema):** Various preparations like *Mahanarayan taila* and *Yashtimadhu taila* etc are administered through anal route. This helps in reducing the severity of attacks.

**1.6.10.2 Shaman chikitsa (Alleviation)**

Commonly used drugs for this therapy are:

**Churna:** Yashtimadhu + tankan, Pushkarmoola churna, Shringyadi churna and Shatayadi Churna

**Aasav:** Kanakasav, Somasav and Dashmoolarishta

**Bhasma:** Abhrak bhasma, Raupya bhasma, Shrung bhasma, Suvarna bhasma and Moti Bhasma

**Ras kalpa:** Brihat Vata Chintamani Rasa, Shwas-kasa chintamani rasa, Suvarna malini vasant, Shwaskuthar Rasa and Nag guti

**Avaleha:** Chyavanprashavleha, Kantkari Avaleha, Vasa Avaleha, Agastiprasha and Chitrak Haritaki Avaleha

**Miscellaneous:** Vardhaman Pimpali and Chaushati Pimpali

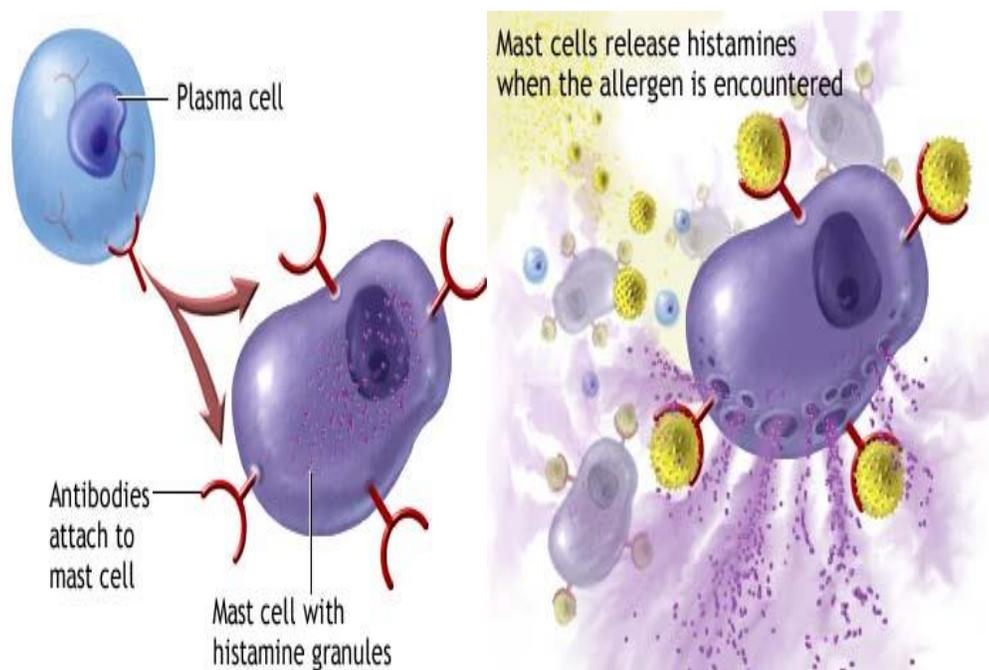
### 1.6.11 Mechanism action of antiasthmatic activity (Tripathi, 2004)

Following approaches are used to treat the asthma

- Prevention of antigen and antibody reaction.
- Neutralization of IgE antibody.
- Suppression of inflammation and bronchial hyperactivity.
- Prevention of release of mediators.
- Antagonism of released mediators.
- Blockade of constrictor neurotransmitter.
- Mimicking dilator neurotransmitter.
- Directly acting bronchodilators.

### 1.7 ALLERGY

Allergy is a disorder of the immune system to overly reactive or hypersensitive individuals to a substance that is tolerated by most other people. During an allergic reaction, some tissue injury occurs. The antigens inducing allergic reactions commonly include certain foods (milk, peanuts, shellfish, and eggs), antibiotics (pencillin, tetracycline), vaccines (pertusis, typhoid), venoms (honey bee, wasp and snake), cosmetics, pollens, dust, molds, iodine containing dyes, chemicals in plants such as poison ivy and even microbes (**Gerard, 2006**).



**Photograph 1.2 Allergen challenged histamine release by mast cells**

### **1.7.1 Allergic reactions and symptoms**

#### **1.7.1.1 Type-I (Anaphylactic) reactions**

These are the most common reactions occurring within a few minutes after a person sensitized to an allergen is re-exposed to it. In response to the first exposure to certain allergens, some people produce IgE antibodies that bind to the surface of mast cell and basophils. The next time the same allergens enters the body, it attaches to the IgE antibodies already present, the antigen-antibody (AG : AB) reaction takes place on the mast cell surface releasing mediators like histamine, 5-HT, LT-C<sub>4</sub> and D<sub>4</sub>, prostaglandins, platelet activating factors etc. resulting urticaria, itching, angioedema, bronchospasm, rhinitis or anaphylactic shock. The manifestations occur quickly after challenge and are called immediate hypersensitivity (**Gerard, 2006**).

#### **1.7.1.2 Type-II (Cytotoxic) reactions**

The cytotoxic reaction are caused by antibodies (IgG, IgM) directed against antigens on a persons blood cells (red blood cells, lymphocytes or platelets) or tissue cells. The reaction of antibodies and antigens usually leads to activation of complement and cytolysis occurs, e.g. thrombocytopenia, agranulocytosis, aplastic anaemia, haemolysis, organ damage (liver, kidney and muscle), and systemic lupus erythematosus (**Gerard, 2006**).

#### **1.7.1.3 Type-III (Retard, Arthus) reactions**

These are mediated by circulating antibodies (predominantly IgG, moping AB). The antigens, antibody (AG: AB) complexes bind complement and precipitate on vascular endothelium giving rise to a destructive inflammatory response which manifest in to rashes, serum sickness (fever, arthralgia and lymphadenopathy), polyarteries nodosa and Stevens-Johnson syndrome (erythema multiforme, arthritis, nephritis, myocarditis, and mental symptoms) and the reaction usually subsides in 1-2 weeks (**Gerard, 2006; Tripathi, 2008**).

#### 1.7.1.4 Type-IV (Delayed hypersensitivity) reactions

Delayed hypersensitivity or cell mediated reaction usually appear 12-72 hrs after exposure to an allergen. These reactions occur when allergens are taken up by antigens presenting cells that migrate to lymph nodes and present the allergen to T cells, which then proliferate. Some of the new T cells return to the site of allergen entry into the body, where they produce  $\chi$ -interferon, which activates macrophages, tumor necrosis factor, which stimulates an inflammatory response. e.g. Contact dermatitis, some rashes, photosensitization (Gerard, 2006; Tripathi, 2008).

#### 1.7.2 Diagnosis and assessment of allergies

There are several methods for the diagnosis and assessment of allergies

##### 1.7.2.1 Skin test

For assessing the presence of specific IgE antibodies, allergy skin testing, is the preferred method compared to *in vitro* tests eg. radioallergosorbent test (RAST) as it is more sensitive and specific, simpler to use, and less expensive.

The typical method of diagnosis and monitoring of Type-I hypersensitivity is skin testing, also known as "scratch testing" and "prick testing" due to the series of pricks and/or scratches made into the patient's skin. Small amounts of suspected allergens and/or their extracts (pollen, grass, mite proteins, peanut extract, etc.) are introduced to sites on the skin marked with pen or dye. The allergens are either injected intradermally or into small scratching made into the patient's skin, often with a small plastic device. Common areas for testing include the inside forearm and the back. If the patient is allergic to the particular substance, a visible inflammatory reaction usually occurs within 30 min. This response is ranging from slight reddening of the skin to full-blown

hives in extremely sensitive patients. After performing the skin test, corticosteroids cream is applied, to the test area to reduce discomfort (itching and inflammation).

#### **1.7.2.2 Blood test**

This kind of testing is also known as a "Total IgE count". In order to qualify type-I hypersensitivity, this method measures the amount of serum IgE contained within the patient's serum. This can be determined through the use of radiometric and colorimetric immunoassays. Even the levels the amount of IgE specific to certain allergens can be measured through use of the radioallergosorbent test (RAST). A leading RAST, which is a commercially available qualitative serological test employed for screening of allergic sensitization in patients with suspected allergic diseases, has a sensitivity of about 70.8% and a positive predictive value of 72.6% according to a large study.

#### **1.7.3 Role of mast cell in allergy**

Mast cells play a key role in the inflammatory process. In activated condition, a mast cell rapidly releases its characteristic granules and various hormonal mediators into the interstitium. Mast cells can be stimulated to degranulate by direct injury (e.g. physical or chemical), cross-linking of IgE receptors, or by activated complement proteins. In allergic reactions, mast cells remain inactive until an allergen binds to IgE already in association with the cell. Allergens are generally proteins or polysaccharides. The allergen binds to IgE molecules on the mast cell surface. It appears that binding of two or more IgE molecules e.g. crosslinking is required to activate the mast cell; the steric changes lead to a slight disturbance to the cell membrane structure, causing a complex sequence of reactions inside the cell that lead to activation of the cell. Although this reaction is most well understood in terms of allergy, it appears to have evolved as a defense system against intestinal worm infestations.

#### 1.7.4 Limitation of asthma and allergy therapy

For asthma and allergy, an allopathic medication has to be prescribed for longer period. Where such treatment produce minor and major adverse or side effects in the body such as nasal congestion, headache, dizziness, rashes, dysuria, nausea, insomnia, cardiac arrhythmia or cardiac arrest, gastric pain, rectal inflammation, convulsion, palpitation, restlessness, nervousness, throat irritation, ankle edema, systemic toxicity, muscle cramps and muscle tremors in dose related manner. Apart, children are more liable to develop CNS toxicity.

**Scope and relevance of study on Ayurvedic formulation:** Considering these problems a direct need is being felt since long for alternative therapy for Asthma and allergy. Fortunately Ayurveda provides a better way of cure of these problems and therefore this aspect should be looked seriously. And a thorough study of Ayurvedic formulations with antiasthmatic / antiallergic potential be concluded so that a safer therapy for asthma and allergy can be developed. One such herbomineral formulation of Ayurveda is *Shwaskuthar Rasa* which is highly valued for treating asthma, allergy and other respiratory disorders. However, on the pretext that as the heavy metals are used in the preparation of such formulations and therefore they are toxic, these formulations have come under a whirl-wind by a report published in JAMA (**Saper et al., 2004**). Fortunately the knowledge of nanotechnology which reveals that after nanosizing, the physical, chemical and biological properties of the materials can be changed entirely and as a result safe material can exhibit toxic symptoms and so-called toxic metals / material can turn into a biologically safe entities. As this revaluation has completely and totally changed the conceptual behavior of the materials in general and metals in particular, the time tested, valued and highly reputed herbomineral formulations of Ayurveda need to be scientifically studied /

investigated thoroughly for their therapeutic efficacy and potency as well as safety/toxicity aspects.

Considering this fact, *Shwaskuthar Rasa* – although prepared using so-called heavy metals needs to be investigated thoroughly for its potency, efficacy as well as for its so-called toxicity in the light of knowledge of nanoscience/nanomedicine as this formulation is prepared by through trituration of metals/materials including mercury and arsenic with herbs leading to the used materials reducing to particle size reaching to nano or near nanoscale.

#### **1.7.5 Herbs reported for treatment of asthma and allergy**

In Ayurveda, the respiratory functions are interrelated with those of other organs that supply nourishment to the body viz. the stomach. It is believed that phlegm, humor or *kapha* (which is one of the three basic humors of the Ayurveda) is produced in the stomach and then accumulates in the lungs. Correcting imbalances in the basic humors is critical to the health and can be achieved through proper digestion and metabolism. There are some plants used in Ayurveda formulations for the management of asthma.

#### **1.8 SAFETY ASPECTS OF HERBOMINERAL FORMULATIONS**

Ayurveda forms an important component of health care system in India. This system is based upon centuries old observations, rich traditional wisdom and with its own strong basic principles and philosophy as its skeleton and body. As per Ayurvedic concepts, every material of earth is made up of five basic components, which are prithvi (earth), jal (water), pawak-tej (fire), vayu (air), gagan (space). This is true to all living forms including plants, animals as well as human beings.







### **1.8.1 Evidence for safety**

#### **1.8.1.1 Traditional use**

Ayurvedic medicines have been traditionally used for thousands of years in Indian subcontinent. In 1998 as per statistics of Govt. of India, there were more than 6 lacs physicians of Indian Systems of Medicine and Homeopathy in India (**Indian Systems of Medicine and Homeopathy in India, 1999**). Out of which more than half belong to Ayurveda stream. Resurgence of Ayurveda has led to the need of its scientific validation both in terms of efficacy and safety for its global acceptance. Appearance of few reports in international journals have further focused the attention and need on safety aspects of certain categories of Ayurvedic products.

About 80% of the population in India depends on traditional system of therapy, out of which almost 75% depend on Ayurvedic medicines in one form or the other, meaning thereby that approx. 2.5 lacs Ayurvedic physicians see on an average treat 10 patients per day amounting to 25 lacs patients per day. Apart almost equal number of people do not visit physicians but use these medicines on their own. This means that overall almost 50 lacs people use Ayurvedic medicines on daily basis in India. Even after such vast usage of a therapy system, the incidences of side effects of the system are almost negligible. This may be considered as the best evidence of safety of Ayurvedic medicines being prescribed by their traditional pattern.

#### **1.8.1.2 Regulatory aspects**

Drugs and Cosmetic Act of India controlling the Ayurvedic medicines has recognized the presence of some toxic substances in Ayurvedic formulations and has given them a separate Schedule (Schedule E1) for listing of such substances. During preparing the formulations containing such ingredients one need to undertake their detoxification called shodhana in Ayurveda as per the ancient text and

all such formulations containing such substances need to carry a warning on their labels as 'to be taken under medical supervision only'. As per Ayurvedic text also the Ayurvedic formulations may contain substances, metals, etc. which, if not used following the Ayurvedic principles, as prescribed may show symptoms of toxicity (**Malik, 1993**).

#### **1.8.1.3 Generally recognized as safe (GRAS) drugs**

Ayurveda uses holistic approach of treatment where food, medicine and non-therapeutic measure like exercise and the way of life one should lead go together. Therefore, it is presumed that all other ingredients except those published in Schedule E1 do fall under the list of GRAS i.e. Generally Recognized and Safe and hence they have not been notified separately.

#### **1.8.1.4 Adverse drug reaction (ADR) monitoring system**

Although there is no formal system of ADR monitoring of Ayurvedic medicines in India, but it has a free press and an active print and electronic media. And so far cases of adverse drug reaction of Ayurvedic medicines prepared as per prescribed procedure have not been reported.

#### **1.8.1.5 Toxicity studies**

Drugs and Cosmetic Act of India control manufacturing of Ayurvedic medicine, under certain conditions, to be met by the manufacturer meet before granting manufacturing permission. Toxicity studies and clinical trials are not mandatory for grant of such licenses and as such there are no guidelines to conduct toxicity studies on Ayurvedic medicines in India. ICMR however, has recently issued preliminary guide-lines (Ethical guidelines for Biomedical Research on Human Subjects) for the same. Several Ayurvedic products have undergone toxicity studies at academic institutions as well as industries in India. Due to want of clear-cut guidelines most of these studies have covered the following aspects:

- Acute toxicity – LD50 in two species
- Sub chronic toxicity – 28 days to 6 months study giving drug through oral route in single species

### **1.8.2 Factors responsible for toxicity of Ayurvedic medicines**

#### **1.8.2.1 Improper manufacturing process**

Certain herbs are inherently toxic and ought to be used after proper shodhana (detoxification) process in the formulations. Kuchla (Nuxvomica) and Vatsanabh (Aconitum species) are such few drug. It not used after due shodhana process as per prescribed text, they may be toxic or some time even lethal. Certain formulations contain metallic ingredients which have to be prepared strictly as per the classical Ayurvedic text which include methods to render them non-toxic or detoxified. Sometimes use of shortcuts by unscrupulous manufactures may raise some safety problems. However, it is equally true that if Ayurvedic drugs are prepared properly following the procedure religiously such problem do not arise. One of such most commonly used Ayurvedic formulation is Swarna Vasant Malti, which contains gold-based mercury and sulphur.

#### **1.8.2.2 Contaminants**

Recently 17 out of 70 Ayurvedic products tested were reported to contain heavy metals (**Saper et al., 2004**) in more than prescribed limits. Other cases they are intentionally added to provide therapeutic efficacy as per the Ayurvedic text labeling them as toxic. The following points need intense scientific debate on this issue.

- Various methods used to test heavy metals involve process of digestion, which converts, bound metal into free metals, which are then tested using analytical tools. Do these products undergo the same process inside the human body and actually release metals from bound to free form is a matter to be ascertained.

- Does mere presence of heavy metals in plant derived drug makes it toxic or the presence of heavy metals in plants may be contributing to therapeutic activity as well?
- In India, we do not have any systematic study base by which the limit of heavy metals can be decided. It is recommend therefore, to first screen the plant materials available for their heavy metal presence from various geographical locations and then decide their limits.

It is also possible that sometimes heavy metals may come as contaminant during processing in improper vessels or from the water used. These are probable sources of contamination and Good Manufacturing Practices should be able to take care of it.

#### **1.8.2.3 Quality of Ayurvedic medicines**

Maintaining the quality of Ayurvedic medicine is of paramount importance. Government of India nearly after almost 30 years of efforts has developed Ayurvedic Pharmacopoeia of India giving the quality standards of certain raw materials. Since Ayurveda medicines cover a large number of ingredients and formulations, generation of quality specifications of all the ingredients and formulations is an uphill task requiring time. Therefore most of the Indian Ayurvedic industries use their own in-house standards to maintain the quality. However, presence of unscrupulous manufactures can't be ruled out. GMP guidelines for Ayurvedic medicines in India have recommended implementations of quality control measures to curve such incidences.

#### **1.8.2.4 Drug abuse**

Charaka Samhita has classified physicians into 3 categories; genuine physician, feigned physician, and pseudo-physician. Due to socio-economic reasons quackery is also prevalent in certain parts of India in the name of Indigenous System practitioners.

In one study, the presence of phenytoin and phenobarbital in the Ayurvedic tablets given to the patients of epilepsy, has been reported (**Gogtay et al., 2002**). Presence of corticosteroids in some of the Ayurvedic preparations prescribed by the so-called traditional medicinal physicians has also been reported (**Gupta et al., 2000**). In the study almost 42% Ayurvedic medicine samples were found to be adulterated with corticosteroids.

**Table 1.5 List of herbomineral formulations**

<b>Ayurvedic product</b>	<b>Manufacturer</b>	<b>Mineral / metal ingredients</b>
Bal Chamcha	Jalaram	Lead
Bala Guti	Zandu Pharma Products	Lead
Bala Sogathi	Navjeevan	Lead and mercury
Balguti Kesaria	Kesari Ayurvedic Pharmacy	Lead and mercury
Gesari	Harinarayan Pharmacy	Lead
Karela	Himalaya	Lead
Maha Sudarshan Churna	Dabur	Lead
Maha Sudarshan Churna	Zandu Pharma Products	Lead, mercury and arsenic
Mahalakshmi Vilas Ras with gold	Baidyanath	Lead, mercury and arsenic
Mahayograj Guggulu with silver and Makardhwaj	Baidyanath	Lead, mercury and arsenic
Navratna Rasa	Unjha Ayurvedic Pharmacy	Lead, mercury and arsenic
Safi	Syncom	Arsenic
Shilajit	Baidyanath	Lead and arsenic
Swarna Mahayograj Guggulu with gold	Jalaram	Lead, mercury and arsenic