3.1 HERBAL INGREDIENTS

3.1.1 *Piper longum*

**Banga et al., (1964)** studies have been carried out to validate the traditional claims of Ayurveda for antiallergic and antiasthmatic activity of *P. longum* fruits extract reduces passive cutaneous anaphylaxis in rats and protects guinea pigs against antigen induced bronchospasm.

**Fernandez et al., (1980)** reported the long-term administration of *P. longum* fruits significantly reduces the frequency and severity of asthma attacks in children of different age groups. In other study pediatric patients with asthma received *P. longum* fruits daily for several weeks all patients showed clinical improvement.

**Dhanukar et al., (1984)** clinical studies have revealed that *P. longum* was very effective in the treatment of bronchial asthma in children.

**Choudhary (2006)** observed the treatment with *P. longum* extract in rats challenged with horse serum along with triple antigen containing *Bordetella pertussis* inhibited degranulation of mast cells.

**Dhanukar et al., (1981)** reported the petroleum ether extract of the *P. longum* fruits antagonized morphine-induced respiratory depression in mice.

**Thakur et al., (2009)** evaluated effects of essential oil of *P. longum* and *A. sativum* on muscular activity of whole *Fasciola gigantica* and its strip preparation.

**Umadevi et al., (2009)** studied the effect of *P. logum* in the brain tissue of experimental group of rats using C6 glioma cells.

**Lee et al., (2008)** identified MAO inhibitors from the fruits of *P. longum* activity-guided fractionation of a methylene chloride soluble extract which led to the isolation of three piperine related compounds-methylpiperate, guineensine and piperlonguminine.
Byeoung et al., (2008) isolated piperlongumine, a pyridone alkaloid from *P. longum* which exhibited a potential inhibitory effect on washed rabbit platelet aggregation induced by collagen, arachidonic acid and platelet activating factor without any inhibitory effect that was induced by thrombin.

Abbas et al., (2007) determined the antibacterial and antifungal activities of various extracts of *P. longum* against using a wide variety of pathogenic bacteria and fungi.

Sheela and Lakshmi (2002) determined antiamoebic activity of roots and fruits of *P. longum* in rats.

Nongyao et al., (2004) studied antiamoebic effects of methanol extract of *P. longum* fruit, *P. sarmentosum* root and *Q. infectoria* nut gall against *Entamoeba histolytica* infecting the caecum of mice.

Young et al., (2002) reported mosquito larvicidal activity of *P. longum* fruit extract against the fourth-in star larvae of Aedes aegypti.

Lee et al., (2001) reported *in-vivo* fungicidal activity of *P. longum* fruits against phytopathogenic fungi, *Pyricularia oryzae*, *Rhizoctonia solani*, *Botrytis cineria*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis*.

Tripathi et al., (1999) reported the *P. longum* fruits for their efficacy against experimental infection of *Giardia lamblia* in mice. *P. longum* showed an specific and non-specific immunostimulatory activity.

Sheela et al., (1996) reported efficacy of the fruits of *P. longum* used in traditional remedies against intestinal distress against experimental caecal amoebiasis in rats.

Pathak and Khandelwal (2007) reported the anti-oxidative, anti-apoptotic and chemoprotective ability of piperine in blastogenesis, cytokine release and restoration of splenic cell population which is suggestive of its therapeutic usefulness in immuno-compromised situations.
Sunila and Kuttan (2004) reported alcoholic extract of fruits of *P. longum* and its component piperine effective as immunomodulatory and antitumor activity.

Zhuang *et al.*, (2009) studied bioassay guided isolation of an ethanol extract of the fruit of *P. longum* yielded piperlongumine, piperine and pipernonaline, as the main antihyperlipidemic constituents.

Singha *et al.*, (2008) evaluated the effect of *P. longum* chloroform extract on TNF-α-induced expression of intercellular adhesion molecule-1 on endothelial cells and on NADPH-catalyzed rat liver microsomal lipid peroxidation.

Parka *et al.*, (2007) reported the inhibitory effects of four acidamides, piperine, pipernonaline, piperoctadecalidine and piperlongumine isolated from the fruits of *P. longum* on washed rabbit platelet aggregation.

Christinaa *et al.*, (2006) evaluated the antifibrotic effect of ethanol extract of the fruits of *P. longum* on liver fibrosis that was assessed by measuring the level of liver hydroxy proline (HP) and serum enzyme levels.

Kyeong *et al.*, (2005) discovered piperlongumine from *P. longum* to inhibit melanin production in melanoma B16 cells stimulated with melanocyte stimulating hormone, protoporphyrin IX and piperlongumine suppressed tyrosinase mRNA expression.

Pradeep and Kuttan (2002) studied the effect of piperine on the inhibition of lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. Simultaneous administration of compound with tumor induction produced a significant reduction in tumor nodule formation.

Kumar *et al.*, (2005) reported isolation and characterization of two active principles from *P. longum* using primary human umbilical vein endothelial cells and evaluated the activities of these compounds on TNF-R-induced expression of cell adhesion molecules.
Sunila et al., (2005) reported the radioprotective property of an ethanolic extract of *P. longum* fruits in Swiss mice. The number of bone marrow cells and esterase positive cells was enhanced by the extract when compared to the radiation exposed control animals.

Parka et al., (2002) isolated two piperidine alkaloids, pipernonaline and piperoctadecalidine from *P. longum* and their activity was determined against five species of arthropod pests.

### 3.1.2 *Piper nigrum*

Parganiha et al., (2011) reported anti-asthmatic activity of *P. nigrum* on acetylcholine induced contraction of goat tracheal chain preparation.

Kim and Lee (2009) reported that oral administration of piperine from *P. nigrum* to mice suppressed and reduced the infiltration of eosinophils, hyper responsiveness and inflammation.

Sawanee et al., (2008) reported the ethanol and water extracts of *P. nigrum* for their anti-allergic activity on antigen-induced β-hexosaminidase released from rat-basophilic leukemia cell line, a tumor analog of mast cell.

Hirata et al., (2008) reported the oral administration of a methanolic extract of *P. nigrum* showed a potent dose-dependent inhibition of dinitrofluorobenzene induced cutaneous reaction in mice.

Ramasamy et al., (2006) investigated the effect of piperine on erythrocyte antioxidant status in high fat diet and antithyroid drug induced hyperlipidemic rats.

Milan et al., (2006) investigated the effect of irradiation on antioxidant properties of black pepper extracts by radical scavenging effect on DPPH radicals by determination of reducing power and content of thiobarbituric acid reactive substances.

Lee et al., (2006) studied the bioassay-guided isolation of chloroform extracts of fruits of *P. longum* and *P. nigrum* using an *in vitro* diacylglycerol acyltransferase inhibitory assay has emerged as a potential therapy for the treatment of obesity and type-II diabetes.
Ruo and Daviesw (1997) investigated the effect of a water extract of *P. nigrum* on spontaneous depolarization, accompanying after potentials and N-methyl-D-aspartate induced depolarization in cortical wedges prepared from genetically epilepsy-prone DBA/2 mice.

Jensen et al., (2006) reported piperine from *P. nigrum* has a biphasic effect upon cytochrome P450 monoxygenase activity with an initial suppression followed by induction. Ethyl acetate extract of *P. nigrum* seeds was tested for insecticidal activity toward adult *Musca domestica* and *Drosophila melanogaster*.

Reddy et al., (2004) isolated pergumidiene and trachyone for the first time from *P. nigrum*. These compounds were active against *Bacillus subtilis*, *Bacillus sphaericus*, and *Staphylococcus aureus* amongst Gram +ve bacteria, and *Klebsiella aerogenes* and *Chromobacterium violaceum* among Gram –ve bacterial strains.

Anith et al., (2003) isolated bacterial antagonists of *Phytophthora capsici* from underground shoot portions of rooted cuttings of black pepper. Screening was done on black pepper shoots for suppression of lesion caused by the pathogen.

Aravind et al., (2010) reported the bacteria isolated from black pepper for suppressing *R. similis*. *In vitro* and *in vivo* screenings were used initially to identify the efficient strains of endophytic bacteria that suppress *R. similis*.

Scott et al., (2008) showed the effectiveness of *Piper* spp. extracts for the control of stored products pests and recently studies have tested the extracts of *P. nigrum*, *P. guineense*, and *P. tuberculatum* against insect pests of the home and gardens.

Jensen et al., (2006) reported ethyl acetate extract of *P. nigrum* as a synergist for the insecticide pyrethrum. The effect of this combination was investigated using cDNA microarray analysis of gene expression profiles in *D. melanogaster*.

Park et al., (2002) reported insecticidal activity of materials derived from the fruits of *P. nigrum* against larvae of *Culex pipiens pallens*, *Aedes*
aegypti, and A. togoi was examined and compared with that of commercially available piperine, a known insecticidal compound from *Piper* species.

**Lee et al., (2008)** isolated the eight alkamides by bioassay-guided isolation of ethanolic extracts of fruits of *P. longum* and *P. nigum* as per spectroscopic analysis they were found to be guineensine, retrofracamide C, pipernonaline, piperrolein B, piperchabamide D, pellitorin and dehydropipernonaline.

**Mun et al., (2007)** studied bioactivity guided fractionation of methanolic extracts of fruits of *P. nigrum* five alkamides were isolated and their structures were elucidated via spectroscopic analysis.

### 3.1.3 Aconitum ferox

**Kumar and Muller (1999)** reported the inhibitory effect on lipid peroxidation of methanolic extracts of *A. ferox* was evaluated using bovine brain phospholipids liposomes as model membranes.

**Kumar et al., (2000)** reported methanolic extracts of *A. ferox* to inhibit the biosynthesis of leukotriene B₄ in bovine polymorphonuclear leukocytes.

**Pallavi et al., (2009)** reported optimizing the extraction, separation and liquid chromatography method with UV detection for the simultaneous quantitative determination of aconitine, solanine and piperine in an Ayurvedic preparation using from *A. ferox*, *S. indicum*, *P. nigrum* and *P. longum*.

**Lee et al., (2007)** reported a new norditerpene alkaloid named 8-O-methylhypaconine along with twelve known alkaloids from the underground parts of *A. ferox*. Among the known alkaloids, two dianthramide glucosides were isolated from *A. ferox*.

**Jampani and Alfred (1994)** reported, from root tubers of Ayurvedic processed and unprocessed *A. ferox*. One known diterpenoid alkaloid vakognavine, and nine known norditerpenoid alkaloids, chasmaconitine, crassicauline-A, falconericine, bikhaeonine, pseudaconine, neoline, senbusine-A, isotaiatizidine and columbianine were isolated.
Purushothaman and Chandrasekharan (1974) reported, from the roots of *A. ferox* four alkaloids with pseudoaconitine as the major constituent and other three minor alkaloids as bikhaconitine, veratroyl pseudoaconine and diacetyl pseudoaconitine.

### 3.1.4 *Zingiber officinale*

Chen *et al.*, (2009) reported the pure phenolic compounds isolated from rhizomes of *Z. officinale* are capable of inhibiting allergic reactions and is useful for the treatment and prevention of allergic diseases.

Hiserodt *et al.*, (1998) reported the 10-gingerol phenolic compounds isolated from fresh ginger rhizomes have most active inhibitor of *Mycobacterium avium* and *Mycobacterium tuberculosis* *in vitro*.

Lakshmi *et al.*, (2010) investigated ethanolic extract of rhizomes of *Z. officinale* on anoxia stress tolerance test in Swiss mice. The animals were also subjected to swimming endurance test to gauge the anti-stress potential of the extract.

El-Sharaky *et al.*, (2009) investigated the efficacy of different doses of ginger extract in alleviating hepatotoxicity in male albino rats.

Ajith *et al.*, (2008) evaluated the nephroprotective effect of aqueous ethanol extract of *Z. officinale* against doxorubicin induced acute renal damage in rat. Serum urea and creatinine levels were evaluated as the markers of renal failure.

Ahmed *et al.*, (2008) reported protective effect of dietary feeding of *Z. officinale* against lindane-induced oxidative stress in male albino rats. Oxidative stress was monitored by estimating the extent of lipid peroxidation, scavenging enzymes superoxide dismutase and catalase and the status of the glutathione redox cycle antioxidants.

Badreldin *et al.*, (2008) reported the main pharmacological actions of ginger and its isolated compounds possess immunomodulatory, anti-tumor, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions.
El-Abhara et al., (2008) evaluated the potential role of ginger extract in modulating the extent and severity of ulcerative colitis, a chronically recurrent inflammatory bowel disease.

Siddaraju and Shylaja (2007) reported ginger-free phenolic and ginger hydrolysed phenolic fractions of ginger has potent inhibitory effect of gastric cell proton potassium ATPase activity and H. pylori growth.

Lantza et al., (2007) reported compounds from rhizomes of Z. officinale used an in vitro test system to test the anti-inflammatory activity.

Shukla and Singh (2007) reported that anticancer properties of ginger are attributed to the presence of certain pungent vallinoids, viz. [6]-gingerol and [6]-paradol, as well as some other constituents like shogaols and zingerone etc.

Afshari et al., (2007) studied the effects of ginger powder on nephropathy induced by diabetes and measured changes in plasma antioxidant capacity and lipid peroxidation.

Shukla and Singh (2007) reported some pungent constituents present in ginger have potent antioxidant and anti-inflammatory activities and some of them exhibit cancer preventive activity in experimental carcinogenesis.

Haghigh et al., (2006) compared the effects of indomethacin and ginger on relieving osteoarthritis pain. A double blind, parallel group clinical trial was designed to evaluate the response of 52 patients with knee osteoarthritis to ginger and indomethacin.


Dias et al., (2006) reported that Z. officinale promising for cancer prevention properties. Its modifying potential on the process of colon carcinogenesis induced by 1, 2-dimethylhydrazine (DMH) was investigated in male wistar rats using the aberrant crypt foci (ACF) assay.

Haksar et al., (2006) demonstrated that Z. officinale possesses antioxidant, radioprotective and neuromodulatory properties that can be
effectively utilized for behavioral radioprotection and for efficiently mitigating radiation-induced CTA in both males and females species.

Ghayur et al., (2005) reported hypotensive, endothelium dependent and independent vasodilator and cardio-suppressant and stimulant effects of ginger aqueous extract.

Sharma et al., (2005) investigated the neurobehavioral protective efficacy of a hydroalcoholic extract of ginger in mitigating χ-radiation induced conditioned taste aversion in Sprague Dawley rats.

Bhandari et al., (1998) studied the effects of ethanolic extract of ginger (200 mg/kg, p.o.) in cholesterol fed rabbits. The marked rise in serum and tissue cholesterol, serum triglycerides, serum lipoproteins and phospholipids were measured.

Hasenohrl et al., (1998) performed to gauge the specificity of the anxiolytic action of Zingicomb (a ginger preparation) with respect to the mixture ratio of the single components in the combination preparation. Two different combinations of Zingiber officinale and Ginkgo biloba extracts were compared with the standard.

Sharma and Gupta (1998) studied the acetone and 50% ethanolic extract of ginger and ginger juice against cisplatin effect on gastric emptying in rats.

Surh et al., (1998) reported that some pungent constituents present in ginger have potent antioxidant and anti-inflammatory effects, and some of them exhibited anti-tumor promotional activity in experimental carcinogenesis.

Hasenohrl et al., (1996) reported effects of the known anxiolytic compound diazepam on the behavior of rats in the elevated plus-maze compared with those of zingicomb, a combination preparation of standardized extracts of Ginkgo biloba and Zingiber officinale.

Ueki et al., (2008) studied the effects of systemic administrations of ginger and its pungent constituent, [6]-gingerol, on resting body temperature in rats.
Effie et al., (2003) reported the ability of 20 pungent constituents of ginger and related substances to inhibit arachidonic acid induced platelet activation in human whole blood.

Bliddal (2000) examined the effect of ginger extract compared to placebo and Ibuprofen in patients with osteoarthritis of the hip or knee in a controlled, double blind, double dummy, cross-over study with a wash-out period of one week followed by three treatment periods in a randomized sequence, each of three weeks duration.

Rahuman et al., (2008) reported the larvicidal activity of a petroleum ether extract of Z. officinale against Aedes aegypti and Culex quinquefasciatus.

Ajith and Hema (2007) reported that the hepatoprotective effect of aqueous ethanol extract of Z. officinale against acetaminophen-induced acute toxicity is mediated either by preventing the decline of hepatic antioxidant status or due to its direct radical scavenging capacity.

Iqbal et al., (2006) reported anthelmintic effect of crude powder and crude aqueous extract of dried ginger in sheep naturally infected with mixed species of gastrointestinal nematodes.

Bhandari et al., (2005) observed that the ethanol extract of Z. officinale can protect the tissues from lipid peroxidation in diabetic rats.

Agarwal et al., (2001) reported insect growth regulatory and anti-feedant activity against Spilosoma oblique and antifungal activity against Rhizoctonia solani by thermally labile zingiber fraction from its diethyl ether extract.

Shati and Fahmy (2009) investigated the role of water extract of ginger and Thymus vulgaris to detoxify the injuries of alcohol abuse on liver and brain of mice.

Goyal et al., (2006) reported that treatment with methanol and ethyl acetate extracts of Z. officinale for 8 weeks produces significant reduction in body weight, glucose, insulin and lipid levels as compared to obese control mice.
Zhou et al., (2006) reported that the volatile oil of ginger significantly inhibited T lymphocyte proliferation, decreased the number of total T lymphocytes and T helper cells in a concentration-dependent manner, but increased the percentage of T suppressor cells to total T lymphocytes in the mice.

Borrellia et al., (2004) evaluated the effect of ginger herbal remedy on the contractions induced by electrical stimulation or acetylcholine in the isolated rat ileum.


### 3.2 MINERAL INGREDIENTS

Heavy metals are chemical elements with a specific gravity of at least five times the specific gravity of water. Some well-known toxic metallic elements with a specific gravity five or more are arsenic (5.7), cadmium (8.65), iron (7.9), lead (11.34) and mercury (13.54) (Lide, 1992).

Although the use of heavy metals such as mercury, lead and arsenic has been warned in some of latest publications specially from West on the so-called pretext that as heavy metals are toxic they should not be used in the therapeutic preparations, there are examples of their use since antiquity which are continue even to-day in different parts of the world as therapeutic agents.

The use of so-called heavy metals has not only been recommended/prescribed in ancient Indian medical system their use as drug/therapeutic agents is also common in other medical system of the world where they are being used for treating various diseases and disorders. This substantiates and justifies the utility, relevance and curative or therapeutic value of the heavy metals in all parts of the globe and in all civilization. This also proves the therapeutic potential of so-called heavy metals.
3.2.1 Parada (Mercury)

Mercury known as quick silver is derived from word Hydrargyrum meaning liquid silver. It occurs in nature chiefly as the red crystalline sulphide called cinnabar. It is also found in small globules disseminated through rocks as an amalgam of silver and gold. All the forms of mercury are not toxic, it exists in four major chemical forms:

(i) Elemental mercury (Hg$^0$) in vapour state is the least toxic form.

(ii) Mercury ion (Hg$^{2+}$).

(iii) Alkylated mercury, mainly monomethylmercury and dimethylmercury are the most toxic of mercury compounds.

(iv) Mercury ligand with sulfide which is extremely insoluble in water and non-toxic (Mitra, 2005).

Although mercury is said to be toxic in any form, the difference lies only in how it is absorbed, and the clinical signs and symptoms, and the response to treat modalities. It has been suggested that Hg can cure all diseases if it is prepared and used properly. However, it prepared improperly, it could cause all kinds of diseases (Suoboda, 1998). It has been considered a marvel drug in Ayurveda, with a long history of being used as a nervine tonic and for restoring normalcy to collapsing patients (Arora et al., 1984). In a pharmacokinetic and biodistribution study of the Ayurvedic preparation - Kajjali by using 203Hg tracer (Subramanian et al., 2003) did not observe any ill effects including in the brain in wistar rats. According to Ayurvedic literature, the toxic effects of mercury are neutralized in the presence of sulfur and studies reveal that adaptogenic effects (growth promoting, rejuvenating, and facilitating the learning process) on the central nervous system in small doses of 15 mg/kg (Vohora et al., 1993). The most renowned of all mercury preparations is Makaradhwaja, which acts as rejuvenator. Siddha Makaradhwaja - a reputed Ayurvedic preparation, contains 85.3% Hg and 14.2% S, totaling to 99.5%, which corresponds to almost perfect stoichiometry of HgS. The elimination of elemental mercury occurs via urine, faeces, exhaled air, sweat and saliva. The pattern of excretion is
dependent on the extent to which elemental mercury has been oxidised to the mercuric ion (US-EPA, 1997).

**Preparation:** Mercury is prepared by roasting cinnabar ore in a current of air in a shaft or revolving furnace and the mercury vapours are condensed in brick lined chambers as per following reaction

\[ \text{HgS} + \text{O}_2 \rightarrow \text{Hg} + \text{SO}_2 \]

Mercury is filtered through chamois leather to remove dirt and dust and shaken with dilute nitric acid to remove metallic impurities like tin, lead, copper, zinc and silver etc (Gurbani and Chaudhry, 2004).

### 3.2.1.1 Physical and chemical properties

Mercury is bright, shining, silvery metallic, heavy liquid at ordinary temperature which is extremely mobile and easily divisible into spherical globules. It readily volatilizes on heating and solidifies at -39°C to white malleable ductile solid and boils at 357°C and has a specific gravity of 13.534 g/ml at 25°C. Easily volatilised on heating and its vapours are colourless and its density is indicative of monoatomic nature of the molecule. Superheated mercury vapour is a good conductor of electricity which radiates light of ultraviolet range.

Mercury forms two series of salts, the mercurous salt derived from Hg₂O (mercurous oxide) and mercuric form derived from HgO (mercuric oxide). Mercury usually tarnishes in air because of the metallic impurities present in it forming mercuric sulphide. At higher temperature mercuric oxide is formed. It is practically insoluble in water and alcohol, however readily soluble in concentrated sulphuric acid (Albert et al., 1995).

### 3.2.1.2 Uses of parada (mercury)

Earlier mercury was used therapeutically as cathartic and parasiticide. It was used in the mild mercurial ointment containing 10% mercury to treat secondary lesions produced on the skin in syphilis (a venereal disease). The ointment has been also employed as a fungicide and parasiticide, particularly in the infestation of body and crab lice.
Table 3.1 List of mercurial preparations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredient</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury + Sulphur</td>
<td>Rasa Sindura</td>
</tr>
<tr>
<td>2.</td>
<td>Mercury + Gold + Sulphur</td>
<td>Makaradhwaja</td>
</tr>
<tr>
<td>3.</td>
<td>Mercury + Arsenic + Mercurous chloride + Sulphur</td>
<td>Malla Sindura</td>
</tr>
<tr>
<td>4.</td>
<td>Mercury + Arsenic + Sulphur</td>
<td>Vatagnikumara Rasa</td>
</tr>
<tr>
<td>5.</td>
<td>Mercury + Arsenic trisulphide + Arsenic + Sulphur</td>
<td>Tala Sindura</td>
</tr>
<tr>
<td>6.</td>
<td>Mercury + Arsenic disulphide + Sulphur</td>
<td>Shila Sindura</td>
</tr>
<tr>
<td>7.</td>
<td>Mercury + Arsenic trisulphide + Arsenic disulphide + Arsenic trioxide + Sulphur</td>
<td>Sameer Pannaga</td>
</tr>
<tr>
<td>8.</td>
<td>Cinnabar + Arsenic trisulphide + Sulphur</td>
<td>Hinguliya Manikya Rasa</td>
</tr>
<tr>
<td>9.</td>
<td>Mercury + Copper + Arsenic trisulphide + Arsenic disulphide + Sulphur</td>
<td>Somnathi Tamra</td>
</tr>
<tr>
<td>10.</td>
<td>Mercury + Tin + Ammonium chloride + Sulphur</td>
<td>Swarnavanga</td>
</tr>
<tr>
<td>11.</td>
<td>Mercury + Sulphuric acid + Rock salt</td>
<td>Rasakarupur</td>
</tr>
<tr>
<td>12.</td>
<td>Mercury + Ferrous sulphate + Rock salt</td>
<td>Rasapushpa</td>
</tr>
</tbody>
</table>

3.2.1.3 Parada (mercury) in Ayurveda

All metals are present in the earth's crust enter our bodies continuously at desired levels through food and water. 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 benefit exists develops. As mercury is most widely used metal in Rasa-Shastra discipline of Ayurveda in therapeutic formulations for human use and therefore it has become essential or rather must to carryout
thorough research to generate relevant data on Ayurvedic formulation containing heavy metals in general and mercury in particular relating to their efficacy, safety and toxicity aspects taking into consideration the information cited in old texts as well as employing modern analytical tools and techniques.

3.2.1.4 Fate of parada in the body

A person can be exposed to mercury from breathing in contaminated air, from swallowing or eating contaminated water or food, or from having skin contact with mercury. Not all forms of mercury easily enter the body, even if they come in contact with it and therefore it is important to know that which form of mercury the person has been exposed to, and by which route i.e. air, food or skin. When swallowed small amount of metallic mercury from a broken oral thermometer, virtually none (less than 0.01%) of the mercury enters the body through the stomach or intestines. Even when large amount of metal mercury (about 204 grams) are swallowed very little entered the body.

3.2.1.5 Absorption of parada (mercury) in the body

By inhalation - 80% mercury is absorbed, following ingestion the absorption is

a. Less than 0.01% for metallic mercury.

b. Less than 10% for inorganic mercury (mercury used in Ayurvedic medicines).

c. More than 95% for organic mercury (methyl mercury).

3.2.1.6 Parada in the blood-stream

Methyl mercury is the form of mercury (parada) most easily absorbed through the gastrointestinal tract (about 95%). After eating the foods contaminated with methyl mercury, the mercury enters bloodstream easily and goes rapidly to other parts of body. Only small amounts of methyl mercury enter the blood-stream directly through the skin, but other forms of organic mercury (in particular dimethyl mercury) can rapidly enter the body through the skin.
3.2.1.7 Safe/toxicity limits of parada

The World Health Organization's (WHO) guidelines maintain that the lowest level of mercury that could possibly be harmful to humans is 5 ppm. This level is based on scientific results from the 1960s that placed the level at which risk begins at 50 ppm for most people; WHO then applied a safety factor of 10, deciding that a level of 5 or less is safe for even the most vulnerable populations.

3.2.1.8 Purification of Ayurvedic mercurial preparations

From scientific perspective these detoxifications do not have anything magical about them. All the described processes lead to the elimination of impurities through mechanical / chemical treatment of the mercury, which is then followed by a prolonged heat treatment. Sulphur is added through which neutralises the toxicity of mercury by forming mercuric sulphide inert compound.

Metals of Ayurveda behave differently than their counter parts in modern medicine.

**Phenomenon of isomerism** - i) Kajjali and Parpati have different actions on the body although both of them are black sulphide of mercury. The difference between them is the Sanskara (processing). The preparation of kajjali does not involve heating while Rasa-Parpati is obtained after heating kajjali. ii) Interestingly patients allergic to modern sulpha drugs do not show allergic reaction when Gandhaka Rasayana – an Ayurvedic sulphur preparation is given (probably the reason of this being in the difference in processing, as sulphur compounds are purified and prepared as per Ayurvedic texts). The daily dosage during an Ayurvedic treatment is about 30-40 mg of mercuric sulphide, which usually is given in combination with processed aconite.

It is believed that metals in Ayurvedic preparations exist in complex ionic form due to unique combination of heat processing and herbal treatments. e.g. Loha Bhasma does not give positive test by routine method of testing for iron i.e. with sodium carbonate, potassium sulphocyanide and potassium ferrocyanide. Nitric acid is required to get
the positive result for the presence of iron. Nitric acid breaks the complex iron radicle in to a simpler radicle.

**Some vital facts about mercury compounds:** (a) Inorganic mercury compounds like mercurous chloride and mercuric chloride are white powders and do not generally vaporize at room temperatures like elemental mercury. If they are inhaled, they are not expected to enter the body as easily as inhaled metallic mercury vapor. (b) When inorganic mercury compounds are swallowed, generally less than 10 % are absorbed through the intestinal tract, therefore considering that 1 gm/kg is fed to rats it is very high dose compared to dose of mercury used in Ayurvedic formulations (As compared to methyl mercury, the total amount of HgS accumulated in the tissues ranges about one five-thousandth of methyl mercury). (c) According to the criteria of the WHO, the weekly dose of mercury that can be tolerated by the body is estimated and the United Kingdom’s Food Standards Agency (FSA) uses the safety standard applied by WHO called the Provisional Tolerable Weekly Intake (PTWI) that allows 3.3 µg/kg/week for the general population and 1.6 µg/kg/week for pregnant and nursing women. (d) Inorganic mercury compounds also do not move as easily from the blood of a pregnant woman to her developing child.

Ayurveda has given supreme significance to the human body and even ascribed special position to god in the human body, which signifies the fact that “How precious the human being are”. Ayurveda probably is the only science which says Purshm Purshm Vikshym. which means characters /prakruti of one person is different from the other or in simple words, no two human beings on this earth are same, therefore unlike allopathic, Ayurvedic physicians prescribe different medicine to the persons suffering from the same/similar disease based on their individual Prakruti (nature), Vikruti (Disease), Dosha – dushya sammurchana (gradation of disease process) and Sroto-dushti (tissues
involved) and that to with proper Anupan (vehicle for medicine) along with Pathya –Apathya (Do’s and Don’ts for diet) details.

Mercury and other metals become toxic only when they exceed a tolerable safe level. Thus billions of people can exist without toxic symptoms at a tolerably low level of heavy metals.

3.2.2 Manahsila (Arsenic disulphide/ Realgar)

Mineral arsenicals have long been used in traditional medicines for various diseases. Arsenic in traditional medicines typically comes from deliberate addition for therapeutic purposes, mainly in the form of mineral arsenicals, including orpiment/haratala (As₂S₃), realgar/manahsila (As₂S₂), and arsenolite (As₂O₃). Inorganic arsenic is now accepted in Western medicine as a first line chemotherapeutic agent against certain hematopoietic cancers (Miller et al., 2002; Evens et al., 2004). Orpiment and realgar are less soluble and poorly absorbed from the gastrointestinal tract, whereas the bioavailability of arsenic trioxide is similar to inorganic arsenic salts such as sodium arsenite. Pharmacological studies show that arsenic trioxide and realgar are effective against certain malignancies. Orpiment and realgar are used externally for various skin diseases (Hede, 2007).

Realgar is frequently included as an ingredient in oral traditional remedies for its antipyretic, anti-inflammatory, antiulcer, anticonvulsive, and anti-schistosomiasis actions. Toxicological studies show that cardiovascular toxicity is the major concern for arsenic trioxide and that the gastrointestinal and dermal adverse effects may occur after prolonged use of mineral arsenicals (Liu et al., 2008).

3.2.2.1 Physical and chemical properties

Manahsila (Arsenic disulphide/Realgar) is orange to red colour, resinous luster, sub-metallic compound. Its crystals are translucent to transparent, crystal system is monoclinic and crystal habits include prismatic striated crystals with a rounded diamond-like cross-section. They are wedge-like dome. It is also found as grains, crusts and earthy masses. Cleavage is good in one direction, fracture is subconchoidal, and
specific gravity is 3.5 - 3.6 g/ml at 25 °C. Streak is orange to orange-yellow. As realgar is unstable in light; the specimens be stored in complete darkness, rarely some specimens fluoresce under UV light and crystals are pleochroic between dark red and orange red.

Manahsila (Arsenic disulphide/ Realgar) structure is analogous to that of sulfur and resembles sulfur in most respects except for colour due to which the name "ruby sulfur" has been assigned to realgar. Realgar structure alternates between sulfur atoms and arsenic atoms producing rings of $\text{As}_4\text{S}_4$. The arsenic atoms affect the structure altering it from sulfur's orthorhombic symmetry to realgar monoclinic symmetry. The heavy metal sulfides are generally insoluble and show little toxic action except through the liberation of hydrogen sulfide which is toxic, as a severe irritant and flammable.

3.2.2.2 Use of arsenicals in traditional medicines

Arsenic has been used as a poison and as a therapeutic since ancient times (Liu et al., 2007). In ancient Chinese medicines, the use of arsenic can be traced back to 200 B.C. in Shen Nong Ban Cao Jing, the first traditional Chinese medicine book. Using a poison to attack another poison or to fight against malignant diseases is a common concept in traditional Chinese medicines (Chinese Pharmacopoeia Committee, 2005). The use of mineral elixir made from the “essence of the five planets,” including arsenic-containing minerals, was thought to give humans perpetual life in Indian Ayurvedic medicines (Kumar et al., 2006). The properties of three major arsenic containing minerals used in traditional medicine include orpiment or yellow arsenic, Arsenikon (Greek) or Cihuang (China), and contains $\text{As}_2\text{S}_3$. Another is realgar, which is also called red arsenic due to its deep red colour, or Xionghuang (China), and contains 90% arsenic disulfide ($\text{As}_2\text{S}_2$). Arsenolite or white arsenic contains largely $\text{As}_2\text{O}_3$. Physicians prescribed arsenicals for external and internal use throughout the 19th century (Miller et al., 2002; Evens et al., 2004).
Arsenic and arsenic salts were key ingredients in antiseptics, antispasmodics, haematinics, sedatives, ulcer, and cancer cures. Arsenical preparations, such as Fowler solution (1% potassium arsenite), are used in the treatment of malignant diseases, such as leukemia, Hodgkin’s disease, pernicious anemia, and nonmalignant diseases, such as psoriasis, pemphigus, eczema, and asthma for centuries (Miller et al., 2002; Evens et al., 2004). Arsphenamine was the standard therapy for syphilis for nearly 40 years before it was replaced by penicillin. Approximately 60 different arsenic preparations have been developed and used during the lengthy pharmacological history of arsenic until their uses were gradually replaced by modern agents (Efferth et al., 2007). Today, hundreds of traditional Chinese medicines still use orpiment, realgar or arsenolite, and realgar alone is included in 22 oral remedies based on Chinese Pharmacopeia Committee (2005). In Indian Ayurvedic medicines, realgar is a major component in bhasmas (Kumar et al., 2006). Arsenic trioxide is now becoming a promising chemotherapeutic agent in Western medicine to treat acute promyelocytic leukemia (APL) and possibly other malignancies (Hede, 2007).

Table 3.2 Natural arsenic-containing minerals in traditional medicines

<table>
<thead>
<tr>
<th>Name</th>
<th>Popular Name</th>
<th>Chemical Form</th>
<th>Traditional or Historical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orpiment</td>
<td>Yellow arsenic (Arsenikon, Cihuang)</td>
<td>As$_2$S$_3$ (Arsenic trisulfide)</td>
<td>Cancer, skin diseases, bald head scab disinfectant, psoriasis antispasmodic.</td>
</tr>
<tr>
<td>Realgar</td>
<td>Red arsenic (Xionghuang)</td>
<td>As$_4$S$_4$; As$_2$S$_2$ (Arsenic disulfide, arsenic sulfide)</td>
<td>Malignancies, skin diseases, sedative antipyretic, ulcers anti-inflammation.</td>
</tr>
<tr>
<td>Arsenolite</td>
<td>White arsenic (Pishi)</td>
<td>As$_2$O$_3$ (Arsenic trioxide)</td>
<td>Fowlers solution for psoriasis, syphilis, cancer (especially leukemia)</td>
</tr>
</tbody>
</table>
3.2.2.3 Bioavailability of manahsila (Realgar)

It is generally assumed that the severity of poisoning is related to the total amount of poison ingested, and assessment of health risk associated with arsenic exposure from human ingestion of traditional medicines has typically based on this belief (Ernst, 2002; Cooper et al., 2007). However, in many cases, a significant portion of some forms of mineral arsenicals are poorly absorbed into the body and thus are unavailable to cause systemic damage. The disposition of these arsenicals in the body depends on various key factors, including solubility, absorption, distribution and excretion.

Realgar (As$_2$S$_2$) in Niuhuang Jiedu Pian, a common preparation for a common cold, has a low solubility in water, and only 4% is bioavailable in physiological gastric juice or intestinal fluid (Koch et al., 2007). The average total arsenic concentration in this preparation is approximately 71 % (i.e., 70,000 ppm), corresponding to 28 mg of arsenic per pill, of which only 1 mg of arsenic finds its way into the blood stream, and 40% of this absorbed arsenic (0.4 mg) is excreted in urine (Koch et al., 2007). Realgar (As$_2$S$_2$) exposure results in various arsenical metabolites in the urine, including MMA, DMA, arsenobetaine, and an unknown metabolite, the level of which peaked at approximately 14 h after ingestion (Koch et al., 2007). In healthy volunteers, 1% of total administered arsenic was found in the urine after repeated doses of Niu-huang Jiedu Pian three tablets, twice a day during a 7-day period (Tang and Wang, 2005). Oral administration of realgar (As$_2$S$_2$) in rats (150 mg/kg, daily for 5 weeks) showed that only a small portion of arsenic was absorbed and reached the blood (45 mg/ml), lung (5.4 mg/g), spleen (5.2 mg/g), or liver (2.9 mg/g) (Tang and Wang, 2005). To overcome the low solubility and poor bioavailability, recently realgar (As$_2$S$_2$) nanoparticles have been prepared by cryogrinding with polyvinylpyrrolidone and SDS, where arsenic solubility was greatly increased compared to crude realgar powder (Wu and Ho, 2006). Realgar nanoparticles showed remarkable increases in bioavailability both in vitro and in vivo. For example, urinary recovery of
arsenic in rats after a single oral administration of realgar nanoparticles (50 mg/kg p.o.) was increased to 70% of the dose compared to 25% when realgar was given in crude powder (Wu and Ho, 2006).

3.2.2.4 Pharmacology of manahsila (realgar)

As per Chinese Pharmacopeia Committee (2005) realgar (As₂S₂) is widely used in the combination with traditional medicines for both external and internal uses. The most common over the-counter preparation Niuhuang Jiedu Pain contains 6.4% realgar, and the bioavailability of arsenic released from this preparation is very low (Koch et al., 2007). The therapeutic uses of these preparations range widely, for common colds, tooth-ache, tonsillitis, asthma, abdominal pains, spasms, sedation, ulcers, heat stroke, coma, and delirium.

The interactions of realgar (As₂S₂) with other herbs or minerals, such as cinnabar (HgS), in many cases are unknown. In this perspective, only the anticancer effects of realgar are briefly discussed. To enhance therapeutic efficacy and reduce adverse effects, physicians of traditional Chinese medicine prescribe the combination formulae of plant species/minerals based on clinical experience, and thousands of such formulae have been recorded (Wang et al., 2008).

Realgar (AS₂S₂) is less toxic compared to arsenic trioxide (As₂O₃) and is used alone or in combination for haematologic malignancies (Lu et al., 2002; Shen et al., 2004). Recently, Realgar-Indigo naturalis formulae have been shown to be very effective against promyelocytic leukemia (Wang et al., 2008). Where realgar acts as the principal component of the formula, whereas plant active ingredients (such as indirubin and trashinone IIA) serve as adjuvant in inducing acute promyelocytic leukemia cell differentiation and the degradation/ubiquitination of promyelocytic leukemia-retinoic acid receptor oncoprotein, enhancing G1/G0 arrest in APL cells through hitting multiple targets, and in intensifying aquaglyceroporin-9 expression and thus facilitating transportation of realgar into APL cells (Wang et al., 2008).
3.2.2.5 Toxicology of manahsila (Realgar)

Realgar is widely used externally and internally in combination with other traditional medicines. Many of these preparations are commercially available in drug stores without prescription, and in general, they are safe with very few reports on their toxicities or adverse effects. However, skin lesions and dermal adverse effects are reported from the long-term use of realgar-containing medicines, such as Chinese preparation - Niu-huang Jiedu Pian (Ernst, 2002; Wang, 2005). In humans chronically taking realgar-containing traditional medicines at higher doses, mild gastrointestinal discomfort may occur; however, no myelosuppression was observed (Lu et al., 2002). The major concern for high dose and long-term realgar treatment in humans is cardiac toxicity, manifested as prolonged QT wave, which is a dose-dependent finding. However, this side effect is tolerable and reversible (Shen et al., 2004).

Liver is a major target organ of long-term arsenic toxicity, and the long-term use of realgar in humans may cause fatty liver; however, neither liver fibrosis nor dysfunction was observed (Qin et al., 2006). When realgar containing Indian medicine Swarnabhasma (gold ash) was administered to mice for 8 weeks, no apparent long-term toxicity (as evidenced by serum aminotransferases, urea and creatine levels, and histopathology) was observed (Mitra et al., 2002). However, the well designed dose and time related toxicology studies are required to critically evaluate the toxicology profiles of realgar containing traditional medicines.

“The dose of the material makes it a poison”, the same concept applies in case of arsenic also, in the evaluation of the toxic effects of mineral arsenicals, these dose and duration of administration. Although mineral arsenicals in traditional medicines are beneficial and even curative of various diseases, it should be kept in mind that “the right dose differentiates a remedy from a poison”. Another important consideration should be to balance the benefit and risk ratio.
3.2.3 Gandhaka (Sulphur)

Sulphur is the chemical element with atomic number 16, represented by the symbol (S). It is an abundant, multivalent non-metal. At normal conditions, sulfur atoms form cyclic octatomic molecules with chemical formula S₈. In nature, sulfur can be found as the pure element and as sulfide and sulfate minerals. Elemental sulfur crystals are commonly sought after by mineral collectors for their brightly coloured polyhedron shapes. Being abundant in native form, sulfur was known in ancient times, mentioned for its uses in ancient Greece, China and Egypt. Sulfur fumes were used as fumigants, and sulfur-containing medicinal mixtures were used as balms and anti-parasitics (Greenwood and Earnshaw, 1997).

Elemental sulfur was once extracted from salt domes where it sometimes occurs in nearly pure form, but this method has been obsolete since the late 20th century. Today, almost all elemental sulfur is produced as a by-product of removing sulfur-containing contaminants from natural gas and petroleum. The element's commercial uses are primarily in fertilizers, because of the relatively high requirement of plants for it, and in the manufacture of sulfuric acid, a primary industrial chemical (Albert et al., 1995).

Sulfur is an essential element for all life, and is widely used in biochemical processes. In metabolic reactions, sulfur compounds serve as both fuels and respiratory (oxygen-replacing) materials for simple organisms. Sulfur in organic form is present in the vitamins biotin and thiamine. Sulfur is an important part of many enzymes and in antioxidant molecules like glutathione. Organically bonded sulfur is a component of all proteins, as amino acids like cysteine and methionine. Disulfide bonds are largely responsible for the mechanical strength and insolubility of the protein keratin, found in outer skin, hair, and feathers, and the element contributes to its pungent odour when burned (Stephen and Cand, 2002).
The biosynthesis of organic sulfur compounds from sulfate takes place mainly in plants and bacteria, whereas the oxidation of these compounds to sulfate is characteristic of animal species. Sulfur is excreted as sulfate, the urinary excretion of sulfate generally reflect input from either inorganic or organic sources (Zlotkin et al., 1982).

Sulfur research on humans has been focused on the role of sulphur containing aminoacids (SAAs), low molecular weight thiols, and disulfides in redox reactions. Although plasma thiols can have pro-oxidant or antioxidant actions depending on the physiological circumstances (Lynch et al., 2000), but are generally considered antioxidants. The sulfur containing amino acids include methionine and cysteine. Methylsulfonylmethane (MSM), an important volatile component in the sulfur cycle, is another source of sulfur found in the human diet. Sulfur is the sixth most abundant macromineral in breast milk (McNally et al., 1991) and the third most abundant mineral determined by percentage of total body weight in an adult (Ziegler, 1996).

![Fig 3.1 Sulphur Cycle showing interrelationship of sulphur compounds](image)

### 3.2.3.1 Physical and chemical properties

Gandhaka (Sulphur) occurs as an odourless and tasteless pale grayish-yellow or pale greenish yellow, soft powder, free from grittyness. It burns with a blue flame with the production of sulphur dioxide. It is insoluble in water and alcohol (90 %), its 1 gm dissolves completely in
about 2 ml of carbon disulphide. Sulphur melts at 115.21 °C, boils at 444.6 °C and sublimes easily. Chemically, sulfur can react as either an oxidant or reducing agent. It oxidizes most metals and several non-metals, including carbon, which leads to its negatives charge in most organosulfur compounds, but it reduces several strong oxidants, such as oxygen and fluorine. Precipitated sulphur contains 99.5 percent of sulphur, which is calculated on the basis of anhydrous sulphur (Albert et al., 1995)

Sulphur is very active element and reacts with most metals and non-metals forming sulphides as below:

\[ \text{e.g; } 2\text{Cu} + \text{S} \rightarrow \text{CuS} \]
\[ \text{Hg} + \text{S} \rightarrow \text{HgS} \]

3.2.3.2 Medicinal uses of gandhaka (sulphur)

Sulphur acts as a good scabicide constituent in sulphur ointment. It is also used in the form of lotions or ointments in the treatment of acne. Precipitated sulphur is preferred in liquid mixtures, as its lighter particles get easily suspended. Being an active parasiticide, treatment of many infections and skin disorders, like ring worm infections, pediculosis, psoriasis, eczema etc contains sulphur. Other well-known uses for this element are in insecticides and fungicides (Mohamed, 2010). Gandhak amla saar contains sulphur for treatment of cough and other respiratory disorders (Jamil et al., 2004). Sulfur containing baths have a long history of use for the treatment of psoriasis, rheumatic pain, and infections, and are still prescribed for asthma by physician in France (Stephen and Cand, 2002).

3.2.3.3 Pharmacology of gandhaka (sulphur)

Purified sulfur has been used as a therapeutic agent to reduce clinical manifestations of a reaction to combined radiotherapy called autosensitization, a type of autoimmunity associated with alternative radiation therapy. Thirty-four women with diagnoses of cervical cancer (stages I and II) when given 0.5-1.0 g of purified sulfur mixed with 0.25 g
of glucose orally in the morning every 2-3 hours before irradiation, a significant decrease in the reaction to therapeutic irradiation was noted in the sulfur group and no side effects were observed (Smirnova, 1991). Because radiation causes damage to DNA through free-radical intermediates, thiols with a net-positive charge may protect against radiation poisoning because they concentrate in the microenvironment of DNA and scavenge free radicals (Pollack, 1996).

3.2.3.4 Toxicology of gandhaka (sulphur)

Adverse effects from topically applied sulfur are uncommon and are mainly limited to the skin (Lin et al., 1988; Kligman, 1975). There are reports of fatalities in infants after its massive external application. In patients with ulcerative colitis (UC), protein or other foods high in sulphur containing amino acids should be used with caution. There is evidence linking protein fermentation and subsequent formation of sulfide in the pathogenicity of this disease. Hydrogen sulfide, sulfide, and thioacetic acid are produced and cause irritation to the colonic mucosa, resulting in possible damage to colonic epithelial cells, and leading to inflammation (Geypens et al., 1997). One study found 96 percent of patients with ulcerative colitis carry sulfate-reducing bacteria in the colon compared to only 50 percent of healthy individuals. In another study, a diet low in sulphur containing amino acids produced an improvement in ulcerative colitis patients (Roediger, 1998).

Organic sulfur compounds are metabolized by the molybdenum-dependent mitochondrial enzyme sulfite oxidase (sulfoxidation). This is also the process that detoxifies sulfite food additives. Sulfite is toxic to the nervous system and molybdenum is necessary for its metabolism to a non-toxic form, since sulfite oxidase contains molybdenum in its active center. Normally, sulfite oxidase metabolizes sulfites to sulfates, which are excreted in the urine or reused by the body. A deficiency in molybdenum or sulfite oxidase may make an individual more sensitive to sulfur containing drugs and compounds (Hendler and Rorvik, 2001).
3.2.4 Tankana (Borax)

**Formula**: \( \text{Na}_2\text{B}_4\text{O}_7.10\text{H}_2\text{O} \)

**Mol. Wt**: 381.40

Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid. Borax is having not less than 99 percent and not more than the equivalent of 103 percent of \( \text{Na}_2\text{B}_4\text{O}_7.10\text{H}_2\text{O} \). It occurs as sodium salt of pyroboric acid in the dried lakes of Tibet and India.

**3.2.4.1 Physical and chemical properties**

Tankana (Borax) occurs as colourless crystalline or white crystalline powder, without any odour but having saline and alkaline taste. It efflorescent in dry air, on ignition - it loses all its water of crystallization.

When heated, it looses part of its water of hydration, and swells to a white porous product. When it is heated to redness, the remainder of the water is also lost, and it fuses to a colourless liquid, which on cooling yields a transparent mass called borax glass or bead. Its aqueous solution is distinctly alkaline to litmus paper and to phenolphthalein, because sodium tetraborate gets hydrolysed by water into sodium metaborate and boric acid, and the former further gets hydrolysed to yield sodium hydroxide and boric acid (Chatwal, 2009). Its 1 gm is soluble in 16 ml of water, 1 ml of boiling water and 1 ml of glycerin however it is insoluble in alcohol.

**3.2.4.2 Medicinal uses of tankana (borax)**

Tankana (Borax) is used externally for eye-washes in 2 % concentration also in wet dressing of wounds. Its action has been more bacteriostatic than bactericidal. The emulsifying action of borax on oils is attributed to the formation of free alkali on hydrolysis. It is also used for softening water and has an astringent action (Chatwal, 2009). It is used during sterilization of surgical instruments, to prevent their rusting. Borax is a component of many detergents, cosmetics, and enamel glasses. It is also used to make buffer solutions in biochemistry, as a fire retardant, as an anti-fungal compound for fiberglass, as an insecticide,
as a flux in metallurgy, a texturing agent in cooking. It is incorporated in some commercial vitamin supplements (Albert et al., 1995).

3.2.4.3 Pharmacology of tankana (borax)

Tankana (Borax) significantly reduces the genotoxic effects induced by low doses of heavy metals. The protective roles of borax occurred with the effectiveness on their anti-oxidant capacity. It could be useful in the development of functional food and raw materials of medicine (Turkeza et al., 2010). The borax and boran compounds decreased malondialdehyde (MDA), DNA damage and the protein carbonyl content (PCO) level in blood, superoxide dismutase (SOD), and catalase (CAT) activity in the kidney. These compounds increased glutathione (GSH) concentration in blood and the vitamin C level in plasma. Demonstration also revealed that borax and boron supplementation in diet decreases lipid peroxidation (LPO), and enhances the antioxidant defense mechanism and vitamin status (Sinan et al., 2010). Borax did not show cytotoxic and genotoxic effects, and on the contrary elevates total antioxidant capacity in erythrocytes. Borax has been shown to protect vanadium-induced DNA damage in vitro. Besides, the frequencies of sister-chromatid exchanges (SCEs), micronuclei (MN) rates and chromosomald aberrations (CAs) in peripheral lymphocytes were significantly decreased by borax compared to controls (Geyikoglu and Turkez, 2008).

3.2.4.4 Toxicology of tankana (borax)

Borax has the toxicity to humans, including reproductive and developmental toxicity, neurotoxicity, and nephrotoxicity. The degree of borax toxicity depends on the dose or concentration. The most sensitive endpoints of borax toxicity are developmental and reproductive toxicity (Murray, 1995). Borax causes irritation of skin and respiratory tract. The gastrointestinal tract, skin, vascular system and brain are the principal organs and tissues affected. It causes nausea, persistent vomiting, abdominal pain, diarrhea, erythematous and rashes, unconsciousness, depression and renal failure in animals (Brockman et al., 1985).
Photograph 3.1 Parada (Mercury)  Photograph 3.2 Manahsila (Arsenic disulphide)

Photograph 3.3 Gandhaka (Sulphur)  Photograph 3.4 Tankana (Borax)
3.3 RESEARCH ENVISAGED AND PLAN OF WORK

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 is 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 suffer from asthma. 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 1,00,000 persons aged 5-34 years (Nichols and Longsworth, 2000).

The number of individuals suffering with allergic illnesses is increasing in the industrialized, as well as in large cities of developed as well as developing countries such as India. Industrial advances have brought about chemical pollution in our surroundings, and patients suffering from allergic diseases such as bronchial asthma and atopic dermatitis are heavily affected. Allergies also have reached high prevalence and incidence in all over the world. About 10% of the population suffers from allergies involving localized reactions to common environmental allergens, such as pollen, animal dander, house dust, foods and specific diseases include urticaria, angioedema, allergic rhinitis, and some forms of asthma. A positive family history of allergy is found in 50% of atopic individuals.

As per Ayurveda the Piper longum has been used in the treatment of respiratory tract infections such as cough, bronchitis and asthma. It is good remedy for treating gonorrhea, tuberculosis, sleeping problems and arthritic conditions, malarial fever, diarrhoea and jaundice (Ghoshal et al., 1996; Umadevi et al., 2009). Piper nigrum cures illness such as lung diseases, liver problems and heart diseases. It is much employed as stimulant, in cholera weakness following fevers, vertigo, coma etc. (Mun-Chual et al., 2007). P. nigrum also used as an alterative in psoriasis, allergy, and atherosclerosis and arthritic diseases. Externally, it is valued
for rubefacient and as a local application for relaxed sore throat, piles and some skin diseases (Lee et al., 2008). *Zingiber officinale* is stimulant and carminative, and used frequently for dyspepsia, rheumatism, nervous diseases, gingivitis, toothache, asthma and diabetes (Wang and Wang, 2005). *Z. officinale* possesses anti-emetic, anti-inflammatory, anticancer, anxiolytic and anti-thrombotic effects (Afzal et al., 2001) and it is also important ingredient to prepare a highly valued formulation of Ayurveda called Trikatu which is again prescribed for treating allergy, asthma and related problems. Similarly *Aconitum ferox* possess antileprosy, anti-rheumatic, anti-inflammatory, expectorant, diuretic and antidiabetic. It is used in the treatment of asthma, gout, skin diseases and neuralgia (Sunil et al., 2000; Alok et al., 2008). It is a well known fact that human body constitute of elements/mineral including metals and non-metals in varying proportions playing vital roles in the overall metabolic functions necessary for survival and existence of life itself. Although some elements are present in the body in small proportion or traces but they key role in enzymatic activity and other important functions. And even slight imbalance in their relative proportion may lead to certain metabolic disruption or disorders. And supplementation of these from out side can rectify these abnormalities and imbalance metabolism. Presence of Magnesium, Copper, Manganese, Zinc, Boron, Selenium, Silver, Mercury, Arsenic, Lead, and Gold has been reported in human body. *A. ferox* has poisons value and Ayurveda was also well aware of the toxic effects of mercury and arsenic. Now the simple question arises that inspite of these well illustrated facts of toxic manifestations of mercury and arsenic, why it was thought important mercury, arsenic and *A. ferox* for preparing herbomineral formulations. Whether it was used to enhance the potency of their existing Trikatu formulation for allergy and asthma? Thus incorporation of metallics/minerals to herbal ingredients seems to be rational as it may increase the potency and thus improve the therapeutic efficacy of the preparation. These improved virtues of the
preparation may be due to early absorption and early onset of action and enhanced bioavailability. And as particle size reduction to a greater extent, increases the surface area for improved and faster absorption and decreased accumulation in body organs and recovering / minimizing or nullifying the toxicity of so-called heavy metals. Although these aspects may be true but they need thorough investigation on scientific lives utilizing the existing available tools and techniques.

Metal-based formulations have been in use since time immemorial for treatment of asthma and allergy. Rasa Shastra, endeavoured to free the entire world from diseases through the used of processed mercury. Rasayana concept has been translated into a hypothesis that Rasayana plants cause a non-specific strengthening of immune response and tolerance to antigenic challenge.

Minerals such as mercury and arsenic are considered toxic but it is equally true that by proper shodhana (detoxification) process, they can be made into wonderful medicines. When mercury is properly prepared, it balances all three doshas (humors) of the body, has a soothing effect and prevents diseases and aging process. In India, the Ayurvedic physician uses 20 % pure herbal preparations, 30 % pure mineral preparations and 50 % herbomineral preparations. This percentage of usage illustrates that there is much value to using mineral preparations.

Most of the medicines are mixture of compounds and because of its synergistic action, toxicity is diminished, thereby increasing bioavailability through the cells of the body. Treating the minerals with herbal juices may lead to reduction in particulate size even up to nano scale further enabling increasing potency. Thus these herbomineral formulations are known to be effective even in low concentration.

Ayurveda also mentions the toxicity of mercury and arsenic, therefore it is noticeable that in-spite of thorough knowledge of toxicity of these substances, so-called heavy metals have been incorporated along with the herbs to prepare the herbomineral formulations in Ayurveda
which was found much more effective compared to formulations prepared from herbal ingredients alone. 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 purely on the pretext that as heavy metals are present in these herbomineral formulation and therefore they are toxic.

On a very straight forward statement made in JAMA (Journal of American Medical Association), the Ayurvedic preparations containing so-called heavy metals have been declared unfit for use. Such a conclusion seems to be immature and thus unfortunate as it lacks serious scientific thoughts without considering the whole herbomineral therapy of high repute and long standing. It would be therefore, better and fitness in the things that before reaching to any such conclusion, thorough research with proper scientific approach is taken up on these categories of formulations.

Review of literature revealed that *Shwaskuthar Rasa* is prestigious herbomineral formulation of Ayurveda used in the treatment of various conditions such as asthma, allergy, cough, laryngitis, tuberculosis, mental disorders, chest burn and heart diseases.

During the preparation of *Shwaskuthar Rasa* – A herbomineral formulation of Ayurveda, ingredients after thorough process of Shodhana (detoxification) are triturated for many hours as per Ayurvedic text existing since time immemorial and the process of trituration related to the size reduction to desired level. As high surface area arising due to particle size reduction enhances the release, dissolution, bioavailability and therapeutic action, the reduction to near nano or nano scale of the content of the *Shwaskuthar Rasa* formulation might improve its effectiveness on experimental animals. Nanosizing of the preparations on one hand may increase the bioavailability and efficacy, and on other hand enhance the release/exit from the system and may reduce the accumulation in organs and thus minimizing or nullifying toxicity.
Therefore, the present study aims to open new vista and develop renewed approach for establishing the effect of nanosizing Shwaskuthar Rasa – A herbomineral formulation of Ayurveda for allergy and asthma and many other respiratory tract related problems considering its safety/toxicity aspects in experimental animals.

The research work was proposed to be carried out on the following steps:

♦ Collection, Identification and authentication of herbs
♦ Collection and Identification of metals and non-metals
♦ Detoxification of herbs, metals and non-metals as per Ayurvedic text
♦ Preparation of formulation as per Ayurvedic text
♦ Particle size reduction of preparation and determination of particle size
♦ Determination of concentration of heavy metals in the formulation
♦ Determination of status / phases of heavy metals in the formulation
♦ Study of chemical bonding / organic molecules and their elemental analysis in formulation
♦ Study of temperature decomposition/exothermic peak in formulation
♦ Pharmacological screening of prepared formulations for asthma and allergy on following models
  • Histamine induced bronchospasm in guinea pig
  • Mast cell degranulation in mice
  • Clonidine induced catalepsy in mice
  • Passive cutaneous anaphylaxis in rats
  • Passive paw anaphylaxis in rats
♦ Toxicity / Safety studies of prepared formulations in experimental animals
♦ Biochemical estimations for safety/toxicity studies of formulations
- Haemotological parameters
- Serum profile of various organs

- Estimation of tissue enzymes for brain and lungs
- Determination for accumulation of mercury and arsenic in different body organs
- Compilation and presenting data.