As per Ayurveda the human body comprises of five components representing earth, water, fire, environment and air. The human body receives minerals and metals from earth and gases such as hydrogen and oxygen and nitrogen from water and air. These elements being integral of the body system are vital for its survival as the absence of these the life can not even be presumed. Present in a very fixed proportion they play vital role in human metabolism and deviation/change in ratio and proportion of these causes metabolic disorders thus developing disharmony and abnormalities and giving rise to diseases.

Supplementation by elements to overcome deficiencies or rectifying their disproportion in the body can reestablish the balance and thus can rectifying the disorder/disharmony leading to normal body function/activities. Thus use of minerals and metals for healthy life is as old as the origin of human being itself. Use of metals/minerals in different traditional systems of the world is prevalent.

As per Chinese system of medication and in other part of the world also preparation containing metals/minerals are in use for the treatment of various diseases. Like wise in Ayurveda, large number of herbomineral formulation such as Agnikumar Ras, Chintamani Ras, Laxmivilas Ras, Smriti-Sagar Ras, Mukta Shouktic Bhasma and Vanga Bhasma etc are frequently used Rasas and Bhasmas of Ayurveda comprises of minerals/metals are used as Antidysenteric, aphrodisiac and tonic. Indicated in acute diarrhoea, dysentery, chronic fever, cardiac tonic, alterative and haematinic. Used in heart diseases, congestion, cardiac failure and dilation of heart. Antipyretic, stomachic and expectorant. Use in pneumonia, typhoid and delirium since time immemorial.

*Shwaskuthar Rasa* – a highly reputed formulation of Ayurveda is valued for respiratory problems including Asthma and Allergy. As mercury and arsenic so-called heavy metals are generally considered toxic present in their formulations hence a look on scientific line was felt
necessary for their usage in the formulation. As the particle size is an important in the formation deciding the bioavailability and hence therapeutic efficacy and finally the fate of formulation in the body deciding its bioaccumulation/excretion and their toxicity or safety, as per plan of the study the effect of nanosizing the *Shwaskuthar Rasa* on its therapeutic potential and toxicity/safety was also investigated.

Asthma - a disease of airways arising at any stage and persisting for years or even for life is characterized by chronic airway inflammation and air-way hypersensitivity to a variety of stimuli and air-way obstruction. It is due to spasms of smooth muscle in the walls of smaller bronchi and bronchioles, developing edema of the mucosa of the air-ways, increasing mucus secretion and thus damaging to the epithelium of air-way. For the purpose of diagnosis increased number of inflammatory cells including eosinophils, basophils, macrophages and lymphocytes can be found in bronchoalveolar lavage fluid from asthmatic patients. Wide variations in air-way resistance produce episodic symptoms of breathlessness, chest tightness, cough and wheeze. Asthma is a result of complex interactions between various mediators, several cells and neural pathways. The immediate stimulus is the release of various mediators from a variety of inflammatory cells including mast cells and macrophages.

Majority of asthma are atopic, by forming IgE antibody on exposure to common allergens. When pathogens or foreign antigens cross the epithelial barriers and establish a local infection, the host mobilizes its defenses and direct antibody to the site of pathogen growth. Mast cells are large cells containing distinctive cytoplasmic granules which contain vasoactive amines, histamine, serotonin, leukotrienes etc. Mast cells are activated via antibody bound to Fc receptors specific for IgE. The immediate consequence of antigen cross linking of IgE displayed on the mast cell surface is degranulation, which occurs within seconds and
releases stored vasoactive amines causing various symptoms of allergy and asthma.

Allergy is a disorder of the immune system to overly reactive or hypersensitive individuals to a substance that is tolerated by most other people. Whenever an allergic reaction takes place, some tissue injury occurs. The antigens inducing allergic reactions commonly include certain foods, antibiotics, vaccines, venoms, cosmetics, pollens, dust, molds, iodine containing dyes, chemicals in plants such as poison ivy and even microbes.

*Rasa Shastra* defining the science of mercury refers to the science of making minerals suitable for the body so that they can be used as medicines. Although minerals such as mercury and arsenic are considered toxic but by proper shodhana (detoxification) they can be turned into wonderful medicines. When mercury is properly prepared, balances all three doshas of the body, has a soothing effect, prevents the disease and aging process in the body.

As per Ayurveda, when compounded with any herb the mercury heightens the medicinal properties of the herb. Further mercury is said to give a firm physique, a stable mind, and is considered to be the destroyer of diseases. Traditional herbomineral formulations have been widely used for thousands of years in many countries. Being the mixture of compounds they exert its synergistic actions, their bioavailability through the cells of the body is increased and their toxicity is also reduced. Treating the minerals with herbal juices may lead to further reduction in particle size enabling its potency. As a result such preparations are known to be effective even in low doses.

Shodhana (detoxification) is the preliminary but most important procedure for metals in Ayurveda to make their free from toxicity, potentiate them to achieve the therapeutic excellence and to make them easily digestible, absorbable and assimilable. Thus shodhana is not only a process of chemical purification but a specific process of addition and
separation which causes physical, chemical, biological and therapeutic changes in the metals.

Nanotechnology - a field of applied science and technology that cuts across many disciplines, including colloidal science, chemistry, applied physics and biology. The electronic properties of solids are altered greatly with reductions in their particles size (Top-down approach). 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. Nanomaterials are nanostructured variants of conventional materials (e.g. metal and metal oxide powder) they are applied as additives in material composites or for the functionalisation of surface. These can be produced by breaking up larger fractions of matter into nanostructured elements (top down approach) through mechanical crushing.

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. Of late preparations like *Shwaskuthar Rasa* along with most Rasas and Bhasmas of Ayurveda have come under whirl-wind of a controversy around world due to their heavy metal presence in these formulations which are supposed to be toxic and thus unfit for use. Such a conclusion seems to be immature and thus unfortunate as it lacks serious scientific thoughts. Further it seems such statement has been made without considering the whole herbomineral therapy of high repute and long standing. The present study was therefore aimed to study the efficacy of *Shwaskuthar Rasa* and also investigate the effect of nanosizing their preparation on bio-efficacy and toxicity / safety aspects.

*Shwaskuthar Rasa* is comprises of herbal ingredients - *P. nigrum*, *P. longum*, *A. ferox*, *Z. officinale* and mineral ingredients - mercury, arsenic disulphide, sulphur and borax. The herbal ingredients were identified and authenticated giving specimen numbers. Metals such as
mercury and arsenic (arsenic form of arsenic disulphide), non-metals as sulphur and borax were identified with specific inorganic radical tests.

Specific shodhana process was carried out as per Ayurvedic text for metals, non-metals and herbs to convert into a suitable and acceptable form. After proper shodhana process percentage yield of parada (mercury), manahsila (arsenic disulphide), gandhaka (sulphur), tankana (borax) and Vatsanabha (A. ferox) was found to be 61.72 %, 98.53 %, 97.15 %, 58.38 % and 98.41 % respectively.

*Shwaskuthar Rasa* formulation was prepared as per Ayurvedic text tested for fineness on Rekhapurnatvatam (finger print test for fineness) and Varitaratavam (floatation test for fineness) tests and finally tested for completion process with Nischandratva (complete loss of luster test), Nisvadutvam (test for not imparting any taste), Avami (test for complete transformation of ingredients), Apunarbhavata (test for completion of process and saturation) and Niruttha (rigorous and stringent test for completion) tests as per Ayurvedic quality parameters.

When evaluated organoleptic parameters of *Shwaskuthar Rasa* showed as black colour, tasteless, fine particulate preparation without any metallic sound with physicochemical characters such as loss on drying (0.32 %), total ash (41.70 %), and acid insoluble ash (15.53 %) were found.

Characterization of *Shwaskuthar Rasa* using modern analytical techniques was done to assess the effect of the processing. The average particle size of *Shwaskuthar Rasa* with Zetasizer and particle shape and surface study with SEM, showed spongy structure of the preparation with irregular particle size lying in the submicron range. These characteristics might be due to organic materials from herbal source used in the formulation, it is clear that nanosize crystallites are agglomerated giving rise to micro sized particle with loss of grain boundaries. These studies confirm that *Shwaskuthar Rasa* is nanocrystallite preparation with submicron sized particle (1.22 µ). This
particle size can be considered as the desired specification of the fineness of the final *Shwaskuthar Rasa* formulation.

Kajjali was prepared by taking equal quantities of shuddha parada and shuddha gandhaka in a porcelain mortar and mixture was triturated continuously for 40 hrs till it attained the required blackish appearance and attained lusterless state. This intermediate form of preparation comprising mercury and sulphur is called Kajjali (mercuric sulfide) of non-metallic components. The XRD diffraction peaks of kajjali and *Shwaskuthar Rasa* were taken to assess the status of mercury and it was observed that the diffraction peaks in the XRD pattern of *Shwaskuthar Rasa* corresponding to mercuric sulfide became sharper and intense compared to Kajjali sample at same time some new peaks also appeared due to mercuric sulfide, which were not present in the Kajjali sample. This observation confirms that the trituration of Kajjali helps in the formation of mercuric sulfide and increases the crystallinity in the formulations. The crystallite size was calculated from XRD pattern following the Scherrer equation. It was notable here the full width at half maximum (FWHM) in case of Kajjali was high compared to the finally prepared *Shwaskuthar Rasa* samples which confirms that the size of the crystallite increases. This is due to trituration process involved in the preparation of the Kajjali. Thus the XRD study concludes the presence of nanocrystalline structure of the formulation.

In addition to the mercury (Hg) and arsenic disulphide (As₂S₂) used in the formulation, some other elements were also expected in the formulation, which enters during the detoxification and trituration process. Chemical composition of *Shwaskuthar Rasa* using EDAX and trace metal composition using ICP-MS was done. Which revealed abundance of Carbon (31.24 %), Nitrogen (12.40 %) and Oxygen (42.63 %) in the formulation was observed which was obviously from the herbs used in the preparation. Calcium (1.62 %) conducive to healthy metabolism and preventive stomach lesions, were also found in the final
product. Sodium (2.37 %) needed for maintaining normal fluid balance was also found in the final product. Sulphur (0.89 %) is additional supplement improving the curative properties of the formulation. Other elements such as H (4.65 %) and Cl (3.46 %) were also found in the formulation. Test for the presence of heavy metals was performed where there presence Hg (0.94 ppm) and As (8.78 ppm) was found well within the safe limits recommended by WHO. Thus, the additional elements present in the formulation are clearly due to herbal ingredients used and so may be called as bioavailable. The present study revealed that the proportion of mercury in Shwaskuthar Rasa does not follow a consistent trend, and some mercury is lost during the preparation through direct trituration process.

FIR spectrum of Shwaskuthar Rasa in the region from 50-400 cm$^{-1}$ was studied. Crystalline mercury sulfide (HgS) showed absorption at 83, 92 and 100 cm$^{-1}$ and their presence in the present FIR spectra indicate that Shwaskuthar Rasa, the mercury is essentially present as mercury sulfide. This observation supports the XRD analysis. FTIR spectrum of Shwaskuthar Rasa in the region from 400-4,000 cm$^{-1}$ was studied. Where presence of fairly sharp peaks at 708, 1080, 1131, 1253, 1346, 1440, 1634, 2930 and 3363 cm$^{-1}$ indicated the presence of the organic compounds in the formulations. These arise due to the usage of herbal ingredients. The presence of appreciable concentrations of C, H, O, and N also suggests the presence of organic molecules in the formulation.

Particle size of the preparation may be attributed to the trituration of detoxified metals, non-metals and herbal ingredients for a long duration which causes the change in their chemical nature. Here, the FT-IR analysis shows the possibility of organic matter in the Shwaskuthar Rasa. This could be due to the formation of organometallic complexes in the formulation. The particle size of the formulation matches well with the colloidal size which is suggestive of the possibility and providing a large surface area for increased absorption of
formulation ingredients. The metal contents act as the carrier of the organic components of herbs. In short, metal ingredients as a carrier for the organic contents of Vatsanabha (*A. ferox*), Maricha (*P. nigrum*), Pippali (*P. longum*) and Adarakha (*Z. officinale*) which established usefulness in treatment of asthma, allergy, cough and inflammation etc. From XRD studies of *Shwaskuthar Rasa* it was concluded that mercury sulfide (HgS) in nanocrystalline range (31-66 nm) form associated with organic molecules probably plays an important role in making it biocompatible and non-toxic at therapeutic dose level. Other elements present in *Shwaskuthar Rasa* act as supplement and help in increasing the efficacy of the formulation.

Particle size of any formulation plays important role in deciding the biological availability, therapeutic potential and its excretion from the system. And reduction in particle size has been witnessed with faster absorption, faster assimilation, and early onset of therapeutic activity due to enhanced surface area followed by faster release from the body system. Although *Shwaskuthar Rasa* prepared by Ayurveda norms attains a reduction in particle size. However as it contains mercury and arsenic is accumulated in the body may cause certain complication and toxicity. Keeping this in mind it was planned to subject the prepared formulation to further particle reduction and to investigate the effect of particle size on therapeutic profile, accumulation in organs and safety/toxicity aspects. Size reduction of prepared *Shwaskuthar Rasa* was done using planetary ball mill.

Already prepared *Shwaskuthar Rasa* was weighed and loaded to a hardened two ceramic bowls containing hardened ceramic balls (5 balls/bowl) inside. The bowls were tightly sealed to prevent and minimize any oxidation of the formulation and it was set at 200 rpm. To follow sample preparation, a accurate quantity of milled products, after scheduled milling time (0, 6, 12, 18 and 24 hrs) was withdrawn and analysed by various modern analytical techniques and measured for
their average particle size with Zetasizer and particle surface with SEM. Average particle size of in-process withdrawn samples of *Shwaskuthar Rasa* was found to be 1220 nm, 829 nm, 574 nm, 216 nm and 92 nm for 0, 6, 12, 18 and 24 Hrs respectively. The data of milling revealed that reduction in particle size of preparation was directly proportional to milling time i.e. more the milling time finer the particles eventually reaching to nano size. The SEM analysis it is clear that nanosize crystallites are agglomerated giving rise to micro sized particle with loss of grain boundaries at O hr after milling at scheduled time interval there is formation of nano sized particles at 24 hrs.

XRD analysis of in-process withdrawn samples of *Shwaskuthar Rasa* revealed progressive broadening of the reflection peaks observed which indicates gradual decrease in the particle size and / or an increase in the internal strains with the milling time. From every diffractogram of in-process withdrawn samples of *Shwaskuthar Rasa*, it was possible to determine the crystallite size. The data of milling effect shown that reduction in crystallite size of in-process withdrawn samples of *Shwaskuthar Rasa* was directly proportional to milling time i.e. crystallite size was decreased with increasing milling time.

The Differential scanning calorimetry (DSC) analysis plot for *Shwaskuthar Rasa* of different particles size showed exothermic peaks in the range of 100-110 °C which could be indicative for the decomposition of sulphur. On further heating sharp peaks were found at 486 °C and 570 °C. This correspond to melting of mercuric oxide and mercuric sulfide respectively (melting temperature 500 °C and 580 °C) present in the withdrawn samples of formulations. Weight loss of samples could be due to the burning away of some attached organic molecules. Thus, DSC analysis supports the presence of mercuric oxide and mercuric sulphide observed major by XRD and FIR analysis and organic matter observed by FTIR analysis.
In the present study, the exothermic heat was released by the ball milled samples which was decreased with increasing milling time. This observation is consistent with an increase in the formation of crystalline during milling. The crystallization kinetics was examined by measuring the areas under the crystallization peaks and considering the fact that the differences in these areas are proportional to the relative amounts of crystalline material present. Effect of milling on DSC pattern revealed that decrease in exothermic peak with increasing milling time. It gave a clear indication that crystallinity of in-process withdrawn samples of *Shwaskuthar Rasa* increased with increasing milling time.

FTIR spectrum of in-process withdrawn samples at scheduled time intervals SWR 1220, SWR 829, SWR 574, SWR 216, SWR 92 of *Shwaskuthar Rasa* in the region from 400-4,000 cm\(^{-1}\), this indicated the presence of the organic components in samples of *Shwaskuthar Rasa*. Which arise from the herbs (*P. longum*, *P. nigrum*, *Z. officinale* and *A. ferox*) used as herbal ingredients of formulation. The milling effect on FTIR spectrum of in-process withdrawn samples of *Shwaskuthar Rasa* was found to be there is no fundamental chemical bonding change (stretching and bending vibrations of organic molecules) in the formulation.

*Shwaskuthar Rasa* is valued for asthma and allergy and other respiratory related problems. The in-process withdrawn samples of *Shwaskuthar Rasa* were subjected to pharmacological evaluation. *In vivo* study using experimental animal model was performed to assess the effect of pulverization/milling of *Shwaskuthar Rasa* for antiasthmatic and antiallergic activities of in-process withdrawn samples of *Shwaskuthar Rasa*.

Bronchial asthma is a chronic inflammatory disease characterized by bronchoconstriction and airway inflammation leading to bronchial hyper responsiveness to various stimuli, involving mast cells, eosinophils and T-lymphocytes. Different agonists like acetylcholine, histamine,
5-hydroxyltryptamine and bradykinin are responsible for contractile responses.

In-process withdrawn samples of different particle sized *Shwaskuthar Rasa* were subjected to histamine induced bronchospasm, clonidine induced catalepsy, clonidine induced mast cell degranulation, passive cutaneous anaphylaxis and passive paw anaphylaxis in experimental animals.

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing air-way hyper responsiveness and bronchial air-way inflammation. Antihistaminic revealed that maximum percentage protection offered by SWR 92 *Shwaskuthar Rasa* sample was 76.09 % for bronchorelaxation compared to control (2.06 %). Other *Shwaskuthar Rasa* samples - SWR 216, SWR 574, SWR 829 and SWR 1220 showed 68.96 %, 56.80 %, 45.26 % and 41.23 % bronchorelaxant activity respectively. Statistical highly significant (p<0.01) bronchorelaxation was shown by SWR 92 followed by SWR 216 and SWR 574 whereas SWR 829, SWR 1220 showed significant level of bronchorelaxation (p<0.05) compared to control.

Clonidine challenge study resulted in significant peritoneal mast cell degranulation in mice. Pretreatment of mice with different particles sized *Shwaskuthar Rasa* resulted in significant reduction in degranulation of mast cells when challenged with clonidine and the percent protection was found to be 33.95 %, 46.71 %, 52.13 %, 64.53 % and 72.42 % for SWR 1220, SWR 829, SWR 574, SWR 216 and SWR 92 respectively. SWR 92, SWR 216 and SWR 574 showed statistical highly significant (p<0.01) whereas SWR 574 and SWR 829 showed significant level of (p<0.05) reduction of degranulation of mast cells in mice.

Clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. Different particle sized *Shwaskuthar Rasa* prevented degranulation of mast cells which was probably by raising the cyclic adenosine monophosphate. It is known
that *Shwaskuthar Rasa* may increase intracellular levels of Cycic AMP relaxes air-way smooth muscles and inhibits the release of autocoids from the tissues and basophils based on fineness dependent.

The effect of milling of withdrawn samples of *Shwaskuthar Rasa* on clonidine induced catalepsy in mice was studied. In mice, Clonidine (1mg/kg, s.c) produced catalepsy remained for 3 hrs. The vehicle treated group has shown maximum duration of catalepsy at 120 min after the administration of clonidine. Highly significant (p<0.01) inhibition of catalepsy was observed with SWR 92 and SWR 216 at 90 min and SWR 574 at 120 min and significant (p<0.05) inhibition of clonidine induced catalepsy with SWR 829 and SWR 1220 at 120 min was also noticed.

The passive cutaneous anaphylaxis test was used to evaluate the anti-allergic activity of different particle sized *Shwaskuthar Rasa* by administering antibody passively by intradermal route in fresh rats. After 48 hrs of fixation of antibodies, Different particle sized *Shwaskuthar Rasa* was administered orally (23 mg/kg) and again antigen along with dye was administered intravenously in rats. The samples - SWR 92 and SWR 216 showed statistically highly significant (p<0.01) results with percentage protection found to be 64.12 % and 56.33 % respectively. The samples - SWR 574, SWR 829 and SWR 1220 showed significant (p<0.05) anti-PCA activity with 50.42 %, 41.82 %, 34.70 % protection was found respectively. The study suggested that different particle sized *Shwaskuthar Rasa* may have capability to block the release of mediators from the mast cells. Appearance of a blue wheel showed the extent of liberation of mediators by mast cell.

Allergy is a chronic inflammatory process occurring due to exposure of allergens resulting in the activation of T-lymphocyte with subsequent release of inflammatory mediators. Studies of withdrawn samples of *Shwaskuthar Rasa* on paw edema in rats. The percentage protection against paw edema volume was calculated and maximum
protection was observed at SWR 92 up to 4 hrs. Samples - SWR 92, SWR 216 and SWR 574 gave highly significant (p<0.01) effect with protection of 67.81 %, 62.06 %, 56.32 % respectively. Samples - SWR 829 and SWR 1220 significantly (p<0.05) inhibited the paw edema with protection of 43.67 % and 31.03 % respectively. In general, decrease in particle size of *Shwaskuthar Rasa* enhances the protection against paw edema.

The method of preparation provided an indication that particle size of *Shwaskuthar Rasa* might be an important factor for its bioavailability and efficacy as antiasthmatic and antiallergic activities in experimental animals were found to be fineness dependant i.e. smaller the particle size of the formulation, better was the therapeutic effect. These finding on particle size based efficacy of the *Shwaskuthar Rasa* is suggestive of the fact that smaller particle size of formulation facilitated the drug to cross the biological barriers thus increasing its bioavailability which may be responsible for its enhanced antiasthma and antiallergic activities.

Safety / toxicity studies of different particle sized *Shwaskuthar Rasa* in albino rats were carried out at 23 mg/kg/day for 28 days. No signs of toxicity and mortality were observed in the treated groups as compared to the control group. The effects of different particle sized *Shwaskuthar Rasa* on the body weight did not show significant difference on weight gain of treated group compared with control group.

Haematological examination showed significantly increased (p<0.05) red blood cells (RBCs) and haemoglobin (Hb) in animals of SWR 92 group whereas white blood cells (WBCs), eosinophils and basophils were significantly decreased (p<0.05) in SWR 92 group compared to SWR 216, SWR 574, SWR 829 and SWR 1220 groups. In addition, compared to control, the number of neutrophils, monocytes and lymphocytes were notably decreased in treated groups. The count was within the normal limits. And the overall considered as normal. Finally the study of
haematological parameter showed that different particle sized \textit{Shwaskuthar Rasa} is safe as no untoward change were observed.

The blood serum examination for liver, kidney and heart was carried out on experimental animals. The study revealed that \textit{Shwaskuthar Rasa} of different particle size did not do any damage to these vital organs. The concentration of SGOT, SGPT, ALP, direct bilirubin and total biliruin were significantly (p<0.05) decreased in SWR 92 whereas concentration of SGOT, ALP and direct bilirubin were significantly (p<0.05) decreased in SWR 216 compared to SWR 574, SWR 829 and SWR 1220 treated groups. In addition concentrations of serum urea, serum creatinine, lactate dehydrogenase and creatinine kinase were significantly (p<0.05) decreased in SWR 92 compared to SWR 216, SWR 574, SWR 829 and SWR 1220 treated group. On the whole these values were within the normal range.

As the main route of elimination of heavy metals through urine and feces, the quantity of these heavy metals present in urine and fecal samples of experimental rats was determined. On evaluating the detection of mercury and arsenic content in urine and fecal samples, the concentration of mercury and arsenic in urine was found significantly (p<0.05) increased in SWR 216 and SWR 92 group compared to SWR 574, SWR 829 and SWR 1220 treated group. The fecal analysis showed concentration of mercury significantly (p<0.05) increased in SWR 216 and SWR 92 treated group. Concentration of arsenic did not show statistically significant difference among treated groups. The study therefore indicates mercury and arsenic present in the preparations are excreted out of the body through urine and feces. The elimination of heavy metals is directly proportional to the fineness of \textit{Shwaskuthar Rasa} formulation.

Toxicity of any type or due to any reason, affect the body metabolism, abnormalities, finally effecting the growth of different body parts. Therefore to evaluate the effect of \textit{Shwaskuthar Rasa} of different
particle size, important body organs were taken into consideration. The study revealed that there were no significant difference between the control and treated group on the organ weights of rats. Haematoxylin and eosin staining was used to visualize and differentiate between tissue components in normal and pathological conditions. Histological observations of liver sections from the control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. In contrast, the SWR 1220 and SWR 829 treated group revealed the moderate damage of the groups; the liver sections showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries. The liver sections of the rats treated with SWR 574, SWR 216 and SWR 92 showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration that was almost comparable to the control group. The study on liver thus revealed that Shwaskuthar Rasa with bigger particle size caused some degenerative changes in liver. However, as the particle size decreased the degenerative changes were reduced gradually finally showing liver with normal architecture.

The histological examination of the hematoxylin and eosin stained kidney of control group animals showed normal architecture of renal tubular epithelial cells and presence of hyaline casts in the tubular lumen. The degenerative change was lessened by SWR 1220 treated group, indicating a partial nephroprotective effect. Kidney sections of the SWR 829 treated group showed damage to renal tubular epithelial cells and presence of hyaline casts in the tubular lumen with inflammatory cells. Glomeruli did not show any changes. Less damage to renal tubular epithelial cells with inflammatory cells was observed with SWR 574. But no remarkable effects in the group treated with SWR 216 and SWR 92 compared with the control group was seen.

The histoarchitecture of cardiac tissue of control group appeared to be normal as there was no visible necrotic damage to the myocytes.
However, moderate myocyte membrane damage, myonecrosis, fibroblastic proliferation and infiltration of inflammatory cells was observed in SWR 1220 and SWR 829 treated group. Scrutiny of cardiac tissue of SWR 574 treated group revealed that there was minimum damage to the myocardium with much reduced myonecrosis and lymphocyte infiltration than SWR 1220 group. And SWR 216 and SWR 92 groups showed a more or less normal without any damage to the myocardium similar to control group.

Histological observations of cerebellum sections from the control group showed normal cellular architecture of an outer cortex of gray matter and an inner white matter. In the gray matter three distinct cell layers were distinguished, an outer molecular layer, an inner granular layer and a central layer of Purkinje cells and the white matter consists of myelinated nerve fibers or axons and also contained dendrites and numerous neuroglia. Cerebellum section of SWR 1220 showed molecular layer with highly vacuolated cytoplasm indicating degenerative changes with decrease in their number or complete disappearance in some of the areas. Other areas showed a progressive degree of febricity and a wavy appearance of nerve fibers. Treatment with SWR 829 and SWR 574 showed purkinje cells with slight shrinkage and reduced in size showing a mild degree of cytoplasmic vacuolation and faintly stained nuclei. They lost their specific “flask shaped” appearance and their cell boundaries appeared rounded. But SWR 216 and SWR 92 treated group, the cerebellum sections revealed remarkable pathological changes neither in purkinje cells, molecular layer, granular layer cells nor in the white matter compared to control group.

Histological findings of lung tissues of control group appeared to be normal as there were no pulmonary alterations such as inflammatory cellular infiltration, bronchiolar associated lymphoid tissue (BALT) hyperplasia and perivasculitis. SWR 1220 and SWR 829 have shown moderate pulmonary alterations such as inflammatory cellular
infiltration, BALT hyperplasia and perivasculitis. SWR 574 shows mild tubular injury, BALT hyperplasia, lymphohistiocytic perivasculitis and bronchoalveolar inflammatory infiltrate. SWR 216 and SWR 92 groups showed a more or less normal damage to the lungs compared with control group.

The investigation indicated that the different particle sized _Shwaskuthar Rasa_ has no serious toxicity to most of the important organs at therapeutic doses. Besides, there were no apparent gross lesions at necropsy and histological examination of liver, kidney, heart, brain and lungs also did not reveal extreme pathological changes at therapeutic dose levels. This might be due to the presence of metallic ingredients in the formulation in oxide/sulphide form which is non-toxic. This is clear from our studies on withdrawn different particle sized _Shwaskuthar Rasa_ samples SWR 216 and SWR 92 of very fine particle size. The studies also revealed that although some abnormalities were observed with _Shwaskuthar Rasa_ of relatively bigger particle size i.e. from SWR 574, SWR 829 and SWR 1220, as the particle size is reduced the abnormalities disappear and cellular structure become near normal. Thus particle size of _Shwaskuthar Rasa_ has a decisive role in toxicity/safety aspects on body system.

Lipid peroxidation - a free radical mediated process, has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals and the uptake of oxygen and rearrangement of double bonds in unsaturated lipids that eventually results in destruction of membrane lipids. Glutathione (GSH) is an important inhibitor of free radical mediated lipid peroxidation.

Administration of different particle sized _Shwaskuthar Rasa_ in rats (23 mg/kg, p.o) for 28 days resulted in a significant (P<0.05) decrease in the lipid peroxidation (LPO) in brain and lungs of SWR 92 treated group. In addition significantly (P<0.05) elevated levels of reduced GSH enzymes in SWR 216 and SWR 92 group of brain and lungs compared
with SWR 574, SWR 829 and SWR 1220 treated groups. With these findings, gives clear indication that *Shwaskuthar Rasa* exhibits antioxidant/tissue protective activity by inhibition of lipid peroxidation and by enhancement of reduced GSH enzymes and, thus can be used in the treatment of toxicity effects where reactive oxygen species are involved. The study revealed a significant decrease in lipid peroxidation and enhanced GSH levels in brain and lungs of rats based on fineness of formulation which suggests that the formulations is protective effect against necrotic changes.

Evaluated the concentrations of so-called heavy metals such as mercury and arsenic in brain and lungs of rats treated with different particle sized *Shwaskuthar Rasa* formulation (23 mg/kg/day, p.o) for 28 days as these metals affect wide range of physiological functions in the body including nervous, excretory, reproductive, respiratory, and haematopoietic systems, if present in exceeded limits. In brain, heavy metals detection showed concentration of mercury significantly (p<0.05) decrease in SWR 92 and SWR 216 groups as well concentration of arsenic was significantly (p<0.05) decreased in SWR 92 and SWR 216 groups. Correspondingly in the lungs, heavy metals detection showed concentration of mercury and arsenic significantly (p<0.05) decrease in SWR 216 and SWR 92 groups compared to SWR 574, SWR 829 and SWR 1220 treated groups. All the observed values for mercury and arsenic found in brain and lungs were within the permissible limits. Our findings also revealed that mercury and arsenic do not accumulate in brain and lungs at abnormal levels, rather they are excreted through urine and feces of rats.

In Conclusion, it was proved that in prepared *Shwaskuthar Rasa* formulation mercury is present in mercury sulfide and mercury oxide forms which are crystalline in nature and is associated with several organic macromolecules derived from the herbal ingredients used as formulation ingredients. In addition, several macro/trace elements are
also found in formulation which seems to be bio-available and possibility responsible for enhancing or adding to the therapeutic profile/level of *Shwaskuthar Rasa*.

Study also revealed that milling of *Shwaskuthar Rasa* decreased its particle size and crystallite size which was clear from in-process withdrawn samples of *Shwaskuthar Rasa*. Increased milling time with increased crystallinity in formulation but there were no changes in chemical bonding of organic molecules present in the *Shwaskuthar Rasa*.

Antiasthmatic and antiallergic activity of in-process withdrawn samples of *Shwaskuthar Rasa* showed enhanced percentage protection against bronchospasm, mast cell degranulation, catalepsy, passive cutaneous anaphylaxis and passive paw anaphylaxis with increasing milling time and thus by the decreasing particle size i.e. antiasthmatic and antiallergic activity based on fineness dependent.

Effect of milling on safety/toxicity of in-process withdrawn samples of *Shwaskuthar Rasa* showed haematological and biochemical parameters of liver, kidney and heart within normal values. The accumulation of mercury and arsenic decreased whereas excretion of mercury and arsenic through urine and feces increased with decreasing particle size of *Shwaskuthar Rasa*.

Finally study indicated enhanced antiasthmatic, antiallergic activity and enhanced safety or reduction in the level of toxicity by decreased accumulation of mercury and arsenic in tissues and by increasing removal of mercury and arsenic through excretory system with decreasing particle size of *Shwaskuthar Rasa*. Further studies on molecular drug receptor mechanism, *in vitro* cell line, gene expression, bioavailability/bioequalance, pharmacokinetic and pharmcodynamic studies on herbomineral formulation - *Shwaskuthar Rasa* a preparation of Ayurveda valued for treatment of asthma, allergy and other respiratory diseases are required.