Use of metals in Ayurveda is perhaps as old as the Ayurveda itself. As per record/literature there are innumerable number of preparations which contain one or more metal as an ingredient in some or other form for treating different diseases / deficiencies. It is not only in India but in other countries as well that the use of metals in therapy is prevalent. The elemental composition of human body also reveals the presence of elements and metals in the body as Oxygen (65 %), Carbon (18 %), Hydrogen (10 %), Nitrogen (3 %), Calcium (1.5 %), Phosphorus (1.0 %), Potassium (0.35 %), Sulfur (0.25 %), Sodium (0.15 %), Magnesium (0.05 %), Copper, Zinc, Selenium, Molybdenum, Fluorine, Chlorine, Iodine, Manganese, Cobalt, Iron (0.70 %) and trace elements as Lithium, Strontium, Aluminum, Silicon, Lead, Vanadium, Mercury, Arsenic and Bromine in negligible amount (Harper et al., 1977).

Although the percentage and proportion of these elements may vary as per need in different organs/parts of body and as per age and stage of development but their presence is vital and must for the existence and survival of any biosystem. Calcium is required in more amount as it is needed mainly for bone and teeth formation. Iron is needed for maintains of haemoglobin level in the blood. Similarly copper, phosphorous, zinc, nickel, manganese, magnesium etc are required in relatively small concentration and lead, arsenic, mercury are needed in still smaller quantities i.e. in trace amounts. Even the analysis of sperm reveals the presence of gold in very small amount probably in ppm for spermatogenesis its role seems to be critical. However it is equally true that a proper balance of elements including metals must be manifested in the body and any disturbance causes disharmony in overall body metabolism functioning causing diseases / disorders. It is thus clear that how important it is to have the presence and proper balance of the minerals and metals for the human body. Thus either to overcome the deficiency of particular element directly or indirectly, the use of metals or minerals as causative agent or as tonic is in use since time immemorial.
As the literature reveals the Ayurvedic physicians were well aware of the virtues and ill effects of elements in general and metals in particular. They knew well the therapeutic efficacy of Iron, Zinc, Copper, Gold, Silver, Mercury, Lead and Arsenic and hence formulations such as Mahalakshmi Vilas Rasa, Mahasudarshan Churna, Mahayogaraja, Makardhwaja and Swarna Mahayogaraja etc containing such ingredients are common. It is however equally important to mention that whenever and wherever these metals were used in medicine they were used after adopting proper shodhana (detoxification) process in religious and manner precisely as per documented text. It is also clearly mentioned that if not used after proper shodhana process they may prove highly toxic causing several side effects. However, when used after proper shodhana process acting as elixir they do wonders as curative agents as well tonics.

The latest concern raises (Saper et al., 2008) about the so-called heavy metal toxicity by herbomineral or organometallic may be indiscussionate.

I. Improper use of so-called heavy metals in the formulation without following proper shodhana process may be the reason as many shodhana process are not only tedious and lengthy but also requiring certain ingredients including herbal ones which either not available in scanty amounts.

II. Thus many pharmaceutical concerns may not be using shodhita (detoxified) ingredients but instead may be using the chemical version of the metal product available in market. Thus instead of using Loha Bhasma from shodita Loha it is quite possible that they may be using iron oxide available in market. Certainly it is not necessary that therapeutic potential bioavailability and further safety / toxicity profile of Loha Bhasma and Iron oxide be same it is only one example however same thing may stand true for mercury, arsenic, lead and zinc as well.

III. The state or status of metal present in formulation is very important. Metal in its original form may be highly toxic as its bio-
accumulation in the body profile may be different. However same metal in different salt forms or in oxide form may exhibit entirely different therapeutic and bioaccumulation profile with little or no side effects.

IV. Finally and more importantly the particle size of the formulation is of paramount significance as the concepts of nanotechnology, nanomaterials and nanomedicines have opened new vistas for understanding the material behaviour. According to nanotechnology concept at nanoscale the physical, chemical, magnetic and biological properties of material may change dramatically and a biologically safe material may turn toxic and on the other hand a toxic material may turn into biologically safe.

Another aspect of nanosizing is more related to toxicity /safety aspects. At nanoscale the so-called heavy metals / then their organometallic complexes can easily enter the cell environment carry out their desired therapeutic effect and with same ease can come-out / exit through flux mechanism out of cell / tissues / organ to be eliminated from system / body thus minimizing the chance of bioaccumulation and hence so-called toxicity or side effects. In the light of above it is therefore essential to carryout the study on such category of formulations of Ayurveda taking in to considerations all the aspects of science before arising to any conclusion.

In a study published in Journal of American Medical Association (JAMA), it was reported that herbal medicine product sold as remedies for treatment of ailments such as arthritis and diabetes contained toxic levels of heavy metals high enough to cause poisoning. It is with this particular idea in mind that the Shwaskuthar Rasa was prepared from ingredients after proper shodhmana process and in-process withdraw samples of different particle size wise subjected to toxicity / safety studies as per prescribed protocol so that the facts are revealed and status is clarified which is necessary from the point of view of health and the global acceptance of herbomineral formulation of Ayurveda in general and Shwaskuthar Rasa in particular.
The heavy metal toxicity occurs because of their accumulation in the tissues/organs. When mercury ingested, 80 % is readily absorbed in the blood through the intestine and distributed in various organs, mainly in the kidneys, brain, lungs, liver, heart and endocrine glands show varying degrees of elevated concentrations where it may be accumulated. The brain is the site of greatest sensitivity for metallic mercury it can readily cross the blood–brain and placental barriers. Thus, mercury can be retained in the brain and fetal tissues (Clarkson et al., 1972). Urine and faeces are main routes for heavy metal exertion from body.

The modern chemical testing might still indicate the presence of metal in the herbomineral formulation but according to Ayurveda, these metals might have been transformed to non-toxic, complex form, which are safe for internal use. These lighter forms of metals contained in herbo-metallic formulations may also work as carriers (yogavahāri), where they are able to carry the herbs (complexed with them) faster to the desired site and start the action immediately. They increase the bioavailability of the herbs to the cell and acting as catalysts. After performing the desired action these harmless light metals are eliminated out of body through our excretory systems (i.e. urine and stool).

As Shwaskuthar Rasa – the herbomineral formulation of Ayurveda consist of so-called heavy metals such as mercury and arsenic and therefore the present study was aimed to carryout the study on haematological, biochemical parameters, body weight, organ weight, tissue enzymes, histology of organs, accumulation and elimination of mercury and arsenic through excretory systems with respect to different particle size of Shwaskuthar Rasa.

**7.1 EXPERIMENTAL ANIMALS**

The procured adult male wistar rats were acclimatized to animal house prior to experimentation. They were randomly divided into different group containing six animals per group, kept in colony cages at ambient temperature of 28° ± 2 ⁰C and 45 to 55 % relative humidity with a 12 hrs light/dark cycle, allowed free access to standard diet and water
ad libitum. The protocol for animal experimentation approved by IAEC (Dr. H. S. Gour Vishwavidyalaya, Sagar) was followed.

7.2 SINGLE DOSE ORAL TOXICITY/SAFETY STUDIES OF IN-PROCESS WITHDRAWN SAMPLES OF SHWASKUTHAR RASA TREATED FOR 28 DAYS

Animal : Rats
Species : Albino
Age/Weight : Adult / 100-150 gm
Sex : Male
No. of animals : 36 (Six animals per group)

Preparation of drug solution: Shwaskuthar Rasa (23 mg/kg, p.o.) suspensions were prepared using 2 % v/v Tween 80.

Group-I treated as control group received 2 % v/v of Tween 80 (10ml/kg, p.o) and animals of Group-II, III, IV, V and VI received Shwaskuthar Rasa samples - SWR 1220, SWR 829, SWR 574, SWR 216, and SWR 92 respectively with dose of 23 mg/kg/day orally for 28 consecutive days.

Body weight of animals was recorded weekly and food consumption and water intake were monitored daily. Albino rats were observed for signs of any abnormalities during the treatment period. At the end of the treatment, animals were fasted overnight but allowed free access to water ad libitum. On 29th day, animals were anesthetized with anesthetic ether and blood samples were obtained by cardiac puncture for haematological and biochemical studies, with and without anticoagulant ethylenediaminetetraacetic acid (EDTA) respectively. After the blood collection, the rats were sacrificed by cervical dislocation and the organs like liver, kidney, heart, lungs and brain were isolated for histological studies.

7.2.1 Body weight

Body weight of the rats was measured weekly during treatment and percentage body weight gain was calculated in control and treatment groups and data recorded (Table 7.1).
Fig 7.1 Effect of different particle sized Shwaskuthar Rasa samples on body weight of rats

7.2.2 Blood analysis

On day 29th all animals were fasted over-night and anesthetized for blood collection from the right ventricle. Blood samples were collected into two tubes: (1) Heparinized centrifuge and (2) Dry non-heparinized centrifuge tubes.

The heparinized blood was used for haematological study which included red blood cells count (RBCs), haemoglobin concentration (Hb), white blood cells count (WBCs) and white blood cells differential count. The non-heparinized blood was allowed to coagulate before being centrifuged and the serum was separated. The separated serum was assayed for serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), direct bilirubin, total bilirubin, creatinine, blood urea nitrogen (BUN), lactate dehydrogenase (LDH) and creatine kinase (CK) concentration and data were recorded.
7.2.2.1 Total RBCs count

The number of red cells in blood were too many and size of the cells was too small. It was therefore not possible to count the cells even under high power. The difficulty was overcome by diluting the blood with RBC diluting fluid to a known degree. The diluted blood was placed in a capillary space of known capacity in between counting chamber and cover slip. The number of the cells in the small capillary space of known volume was then counted under the high magnification power of microscope. The count was calculated by multiplying the number with the dilution factor and was reported as cells per million mm of blood.

7.2.2.2 Total WBCs and differential WBCs count

The number of white blood cells in blood were too many and the size of the cell as also small. Which made impossible to count the cells even under high power. This difficulty was overcome by diluting the blood with a WBC diluting fluid to known degree. The diluted blood was placed in a capillary of known capacity in between a special ruled slide (counting chamber) and a cover slip. The cells were thus spread out in a single layer in capillary space and the number of cells were counted under low magnification power of microscope. The total count was calculated by multiplying with dilution factor and were reported as cells per cubic mm of blood. For both total WBCs count and differential WBCs count, principle and method are similar however, it differ in the composition of WBCs staining fluid.

7.2.2.3 Haemoglobin (Hb) estimation

The collected blood was mixed with N/10 HCl to haemolyze RBCs to liberate haemoglobin. This Hb was converted into acid hematin which was reddish brown in colour. The solution was diluted with distilled water till it matched with standard glass tubes for comparison. The Hb % was directly read from graduated tube (Goyal, 2006).
Fig 7.2 Effect of different particle sized *Shwaskuthar Rasa* samples on RBCs count

Fig 7.3 Effect of different particle sized *Shwaskuthar Rasa* samples on haemoglobin
Fig 7.4 Effect of different particle sized *Shwaskuthar Rasa* samples on WBCs count

Fig 7.5 Effect of different particle sized *Shwaskuthar Rasa* samples on eosinophils
Fig 7.6 Effect of different particle sized *Shwaskuthar Rasa* samples on basophils

Fig 7.7 Effect of different particle sized *Shwaskuthar Rasa* samples on neutrophils
Fig 7.8 Effect of different particle sized *Shwaskuthar Rasa* samples on monocytes

Fig 7.9 Effect of different particle sized *Shwaskuthar Rasa* samples on lymphocytes
7.2.2.4 Assessment of liver function parameters

a) **Serum glutamate oxaloacetate transaminase (SGOT):** SGOT catalyzed the transfer of amino group between L-Aspartate and α-Ketoglutarate to form oxaloacetate and glutamate. The oxaloacetate formed reacts with nicotinamide adenine dinucleotide hydride (NADH) in the presence of malate dehydrogenase (MDH) to formed nicotinamide adenine dinucleotide (NAD). SGOT activity was determined by measuring the rate of oxidation of NADH to NAD at 340 nm (Reitman and Frankel, 1957).

\[
\text{AST} \quad \begin{align*}
\text{L-Aspartate} + \alpha\text{-Ketoglutarate} & \rightarrow \text{Oxaloacetate} + \text{L-Glutamate} \\
\text{MDH} & \\
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ & \rightarrow \text{Malate} + \text{NAD}^+ + \text{H}_2\text{O}
\end{align*}
\]

b) **Serum glutamate pyruvate transaminase (SGPT):** SGPT catalyzed the transfer of amino group between L-Alanine and α-Ketoglutarate to formed pyruvate and glutamate. The pyruvate formed reacts with nicotinamide adenine dinucleotide hydride (NADH) in the presence of lactate dehydrogenase (LDH) to formed nicotinamide adenine dinucleotide (NAD). The rate of oxidation of NADH to NAD was measured at 340 nm (Reitman and Frankel, 1957).

\[
\text{ALT} \quad \begin{align*}
\text{L-Alanine} + \alpha\text{-Ketoglutarate} & \rightarrow \text{Pyruvate} + \text{L-Glutamate} \\
\text{LDH} & \\
\text{Pyruvate} + \text{NADH} + \text{H}^+ & \rightarrow \text{Lactate} + \text{NAD}^+ + \text{H}_2\text{O}
\end{align*}
\]

c) **Alkaline phosphatase (ALP):** The ALP-Tris/Carbonate method is a modification of the Bessey, Lowry and Brock method using p-nitrophenolphosphate as a substrate. Under the optimised conditions ALP present in the sample catalysed the following transphosphorylation reaction.

\[ \text{ALP} \quad \text{p-Nitrophenylphosphate + H}_2\text{O} \rightarrow \text{p-Nitrophenoxide + Phosphate} \]

\( \text{Mg/Alkaline pH} \)

At the pH of the reaction p-nitrophenoxide has an intense yellow colour. The reaction was monitored by measuring the rate of increase in absorbance at 405 nm which is proportional to the activity of ALP in the serum (*King, 1965*).

d) **Direct and total bilirubin:** Bilirubin reacted with diazotized sulfanilic acid to produce azobilirubin, which has an absorbance maximum at 560 nm in the aqueous solution. The intensity of the colour produced was directly proportional to the amount of direct bilirubin concentration present in the sample. The subsequent addition of methanol accelerates the reaction of unconjugated bilirubin in the serum, and a value for total bilirubin was obtained after 5 min. The total bilirubin value represents the sum of the bilirubin glucuronide (direct) and the unconjugated (indirect) bilirubin. The colour produced was measured at 560 nm proportional to the amount of the total bilirubin concentration present in the sample (*Malloy and Evelyn, 1937*).
Fig 7.10 Effect of different particle sized *Shwaskuthar Rasa* samples on SGOT, SGPT and ALP of liver function parameters of rat serum.

Fig 7.11 Effect of different particle sized *Shwaskuthar Rasa* samples on direct and total bilirubin of liver function parameters of rat serum.
7.2.2.5 Assessment of kidney function parameters

a) Blood urea nitrogen (BUN): Urea was catalytically converted to ammonium carbonate by the use of urease. The reaction rate was dependent upon the concentration of the influence of glutamate dehydrogenase (GLDH). The rate of this second reaction was dependent upon the first and measured by the rate of conversion of nicotinamide adenine dinucleotide hydride (NADH) to nicotinamide adenine dinucleotide (NAD) by the change of absorbance at 340 nm (Varley and Alan, 1984).

\[
\text{Urea} + \text{H}_2\text{O} \rightarrow 2\text{NH}_3^+ + \text{CO}_2
\]

\[

\text{GLDH}
\]

\[
2\text{-Oxoglutarate} + \text{NH}_3^+ + \text{NADH} + \text{H}^+ \rightarrow \text{L-Glutamate} + \text{NAD}^+ + \text{H}_2\text{O}
\]

b) Serum creatinine: Picric acid in an alkaline medium reacted with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed measured was directly proportional to the amount of creatinine present in the sample and the absorbance was read at 490-500 nm (Varley and Alan, 1984).

\[
\text{Creatinine} + \text{Alkaline picrate} \rightarrow \text{Orange coloured complex}
\]

### Table 7.4 Effect of different particle sized Shwaskuthar Rasa samples on kidney function parameters of rats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Biochemical parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum urea (mg/dl)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 ml(^a)</td>
<td>20.36 ± 1.04</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>27.12 ± 1.12</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>24.54 ± 1.28</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>22.30 ± 1.16</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>21.38 ± 1.26(^*)</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>19.74 ± 1.18(^*)</td>
</tr>
</tbody>
</table>

\(^a\) Tween 80 (10ml/kg, p.o). The data expressed are mean ± S.E.M. n=6; \(^*\)p<0.05 - significant
7.2.2.6 Assessment of heart function parameters

a) **Serum creatine kinase (CK):** Creatine kinase catalyzed the conversion of creatine phosphate and adenosine diphosphate (ADP) to creatine and adenosine triphosphate (ATP). The ATP and glucose was converted to ADP and glucose-6-phosphate by hexokinase (HK). Glucose-6-phosphate dehydrogenase (G-6-PDH) oxidized at the D-glucose-6-phosphate and reduced the nicotinamide adenine dinucleotide (NAD). The rate of NADH formation was measured at 340 nm is directly proportional to serum creatinine kinase activity ([Faulker and Meites, 1982](#)).

\[
\text{CK} \\
\text{Creatine phosphate + ADP} \rightarrow \text{Creatine + ATP} \\
\text{HK} \\
\text{ATP + D-Glucose}^+ \rightarrow \text{Glucose-6-phosphate + ADP} \\
\text{G-6-PDH} \\
\text{Glucose-6-phosphate + NAD} \rightarrow \text{6-Phosphogluconate + NADH + H}^+ \\
\]
b) Serum lactate dehydrogenase (LDH): LDH catalyzed the oxidation of lactate to pyruvate in the presence of nicotinamide adenine dinucleotide (NAD), which is subsequently reduced to nicotinamide adenine dinucleotide hydride (NADH). The rate of NADH formation was measured at 340 nm which was directly proportional to serum LDH-L activity (Buhl and Jackson, 1978).

\[
\text{LDH} \quad \text{L-Lactate} + \text{NAD} \rightarrow \text{Pyruvate} + \text{NADH} + H^+ 
\]

Table 7.5 Effect of different particle sized *Shwaskuthar Rasa* samples on heart function parameters of rats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Biochemical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lactate dehydrogenase (IU/L)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 ml(^a)</td>
<td>245.28 ± 3.78</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>276.46 ± 4.01</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>264.08 ± 3.95</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>253.52 ± 3.56</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>247.06 ± 3.69 *</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>238.12 ± 2.85 *</td>
</tr>
</tbody>
</table>

\(^a\) Tween 80 (10ml/kg, p.o). The data expressed are mean ± S.E.M. n=6; *p<0.05 - significant

**Fig 7.13** Effect of different particle sized *Shwaskuthar Rasa* samples on various heart function parameters of rat serum
7.2.3 Urine and faecal analysis for mercury and arsenic content

a) Collection of urine and faecal samples: On 28th day, 16-18 hr prior to blood collection, from each group of rats were shifted to plastic metabolic cages for collection of urine and faecal samples. Samples of these urine and faeces were assessed for heavy metals i.e. mercury and arsenic contents and reported as parts per million (ppm).

b) Estimation of heavy metals in urine: Samples of rat urine from each group were transferred to a Teflon container and nitric acid was added (1:1.5). The container was closed for digestion in a stove thermostatically controlled at 90 ± 2 ºC for 4 hr to dissolve the urine samples (Paula et al., 2009). After cooling to room temperature, the digested solution was transferred to a decontaminated tube. The analyses of mercury and arsenic content were carried out by inductively coupled plasma mass spectrometer (ICP-MS, Perkin-Elmer ELAN-6000).

**Table 7.6 Effect of different particle sized Shwaskuthar Rasa samples on mercury and arsenic content in urine of rats**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Heavy metal content in urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mercury (ppm)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 ml&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>0.268 ± 0.021</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>0.312 ± 0.024</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>0.359 ± 0.023</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>0.397 ± 0.026*</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>0.424 ± 0.028**</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tween 80 (10ml/kg, p.o), ppm - parts per million

The data expressed are mean ± S.E.M. n=6; *p<0.05 - significant; ** p<0.01 - highly significant
**Fig 7.14** Effect of different particle sized *Shwaskuthar Rasa* samples on mercury and arsenic content in urine of rats

**c) Estimation of heavy metals in faeces:** Collected samples of rat faeces from each group were dried at 60 °C to a constant weight. The dried faecal samples were dissolved in a mixture of concentrated nitric acid–perchloric acid 4:1 (*Sawicka-Kapusta et al., 1987*). The analyses of mercury and arsenic content were carried out by inductively coupled plasma mass spectrometer (ICP-MS, Perkin-Elmer ELAN-6000).

**7.2.4 Organ weights of experimental rats**

On the last day of oral administration of different particle sized *Shwaskuthar Rasa* samples the rats were decapitated and then organs such as liver, kidney, heart, lungs and brain were isolated and washed with alcohol to remove the blood contents then organ weights were measured with electronic balance.
### Table 7.7 Effect of different particle sized *Shwaskuthar Rasa* samples on mercury and arsenic content in feces of rats

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Concentration of heavy metals in feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mercury (ppm)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 ml&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>0.517 ± 0.025</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>0.545 ± 0.028</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>0.663 ± 0.031</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>0.692 ± 0.022&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>0.784 ± 0.034&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tween 80 (10ml/kg, p.o), ppm - parts per million

The data expressed are mean ± S.E.M. n=6; *p<0.05 - significant

---

**Fig 7.15** Effect of different particle sized *Shwaskuthar Rasa* samples on mercury and arsenic content in feces of rats
Fig 7.16 Effect of different particle sized *Shwaskuthar Rasa* samples on organ weight of rats

7.2.5 Histological study of experimental rats

Two animals from each group were sacrificed on the day of blood withdrawal and organs were isolated. Small pieces of liver, kidney, Brain, lung and heart were taken and tissue specimen from control and treated group were collected and stored in formalin. After usual processing 6 μm thick sections were cut and stained with haematoxylin and eosin and observed by microscope under 200 magnifications ([McManus and Mowry, 1965](#)). All the sections of the tissues were examined under microscope for analyzing the altered architecture of the liver, kidney, lungs, brain and heart tissue due to heavy metal contents in the formulation and improved tissue architecture due to nanoscale particle size of formulation.
**Dehydration:** Tissues were cut into pieces and placed in absolute alcohol. With increasing concentration three changes were given in absolute alcohol each for 15 min.

**Preparation of tissues for embedding:** After three changes in alcohol, pieces of samples were transferred in a mixture of absolute alcohol of xylene (1:1) for 15-20 min. Then mixture was concentrated off and pieces were put in xylene for 30 min and after 30 min of scraping wax were added to the xylene up to saturation for 24 hrs.

**Paraffin infiltration and embedding:** The matured wax was filtered to remove any suspending particles and it was kept in molten state for 24 hrs at 62 °C - 64 °C. The material was transferred directly in molten wax in the first infiltration for 45 min at 62 °C in oven. After the first embedding, tissue pieces were removed and placed in second infiltration and were kept as such at controlled temperature.

**Block preparation:** The lid of cuffing jar was applied on upper and side surface of lid. The filtered matured was poured in the lid up to 4/5th of the total height. The tissues were removed immediately from the infiltration pan and placed gently onto the lid. The lid was placed in a tray containing water. It was kept such that the block separated and floated in the water. The block was cut and trimmed to remove excess wax.

**Microtomy:** The blocks were then cut into ribbons of section with the help of microtome. The ribbon sections were transferred to a slide on which a fixative (egg albumin solution) had been applied previously.

**Staining of slides:** The sections on slides were dewaxed with xylene. Aqueous haematoxylin and alcoholic eosin were used for staining. The section were dehydrated with canada balsam on the slides carefully with glass rod, covered with cover slip, viewed and photographed.
7.2.6 Estimation of tissue enzymes in lung and brain of rats

Lungs and brains from experimental animals were isolated, cut into smaller fragments, weighed and washed separately with 0.9 % NaCl plus EDTA and homogenized in ice-cold 20 mM Tris–HCl buffer (pH 7.4) in an ultra trux tissue homogenizer (1 gm tissue in 10 ml of buffer). The homogenates were centrifuged at 10,000×g at 4 °C for 20 min using Remi C-24 high speed cooling centrifuge (Eugenia Murawska et al, 2011). The clear supernatants were decanted and used to estimate lipid peroxidation (TBRS) and glutathione (GSH) levels.

7.2.6.1 In vivo estimation of tissue lipid peroxidation (LPO)

Thiobarbituric acid reactive substances and malondialdehyde have been used as biomarkers of lipid peroxidation for more than 30 years (Lykkesfeldt, 2007). TBARS was measured by spectrophotometric detection.

**Stock solution of TCA-TBA-HCl reagent:** 15 % w/v tri-chloroacetic acid, 0.375 % w/v thiobarbituric acid, 0.25 N hydrochloric acid were prepared. These solutions were mildly heated to assist in the dissolution of the thiobarbituric acid.

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation. 1.0 ml of supernatant sample (0.1 – 2.0 mg of membrane protein or 0.1-0.2 µmol of lipid phosphate) was combined with 2.0 ml of TCA-TBA-HCl and mixed thoroughly. The solution was heated for 15 min in a boiling water-bath. After cooling the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample was determined at 535 nm using spectrophotometer (Shimadzu). The malondialdehyde concentration of the sample was calculated by using an extinction
coefficient of 1.56 X 105 M⁻¹cm⁻¹ (Beuge and Aust, 1978). The LPO values were expressed as nmol/mg of tissues.

7.2.6.2 In vivo estimation of tissue glutathione (GSH)

Tissue glutathione measurements were performed using a modification of Ellamn et al., 1959. 0.5 ml of supernatant from tissues homogenize was added to 2 ml of 0.1M disodium hydrogen phosphate solution (pH 7.4) and colour was developed by adding 0.2 ml solution of 5, 5-Dithiobis (2-nitrobenzoic acid). Absorbance was read at 412 nm using spectrophotometer (Shimadzu). The glutathione (GSH) values were expressed as µmol/mg of tissues.

Table 7.9 Effect of different particle sized Shwaskuthar Rasa samples on tissue enzymes of rat brain

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Brain tissue enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LPO (nmol/mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 mlᵃ</td>
<td>16.12 ± 0.67</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>25.42 ± 0.45</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>22.86 ± 0.61</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>19.05 ± 0.37</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>17.43 ± 0.41</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>15.89 ± 0.52*</td>
</tr>
</tbody>
</table>

ᵃ Tween 80 (10ml/kg, p.o). The data expressed are mean ± S.E.M. n=6; *p<0.05 - significant; ** p<0.01 - highly significant
Fig 7.17 Effect of different particle sized *Shwaskuthar Rasa* samples on LPO of rat brain

Fig 7.18 Effect of different particle sized *Shwaskuthar Rasa* samples on GSH of rat brain
Table 7.10 Effect of different particle sized *Shwaskuthar Rasa* samples on tissue enzymes of rat lungs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Treatment Group</th>
<th>Particle size of preparation (nm)</th>
<th>Dose mg/kg p.o</th>
<th>Lung tissue enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LPO (nmol/mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>10 ml&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.79 ± 0.53</td>
</tr>
<tr>
<td>II</td>
<td>SWR 1220</td>
<td>1220</td>
<td>23 mg</td>
<td>21.39 ± 0.40</td>
</tr>
<tr>
<td>III</td>
<td>SWR 829</td>
<td>829</td>
<td>23 mg</td>
<td>16.15 ± 0.51</td>
</tr>
<tr>
<td>IV</td>
<td>SWR 574</td>
<td>574</td>
<td>23 mg</td>
<td>15.40 ± 0.39</td>
</tr>
<tr>
<td>V</td>
<td>SWR 216</td>
<td>216</td>
<td>23 mg</td>
<td>14.33 ± 0.36</td>
</tr>
<tr>
<td>VI</td>
<td>SWR 92</td>
<td>92</td>
<td>23 mg</td>
<td>12.48 ± 0.41</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tween 80 (10ml/kg, p.o). The data expressed are mean ± S.E.M. n=6; *p*<0.05 - significant; **p*<0.01 - highly significant

Fig 7.19 Effect of different particle sized *Shwaskuthar Rasa* samples on LPO of rat lungs
Fig 7.20 Effect of different particle sized Shwaskuthar Rasa samples on GSH of rat lungs

7.2.7 Determination of accumulation of mercury and arsenic in rat organs

Concentrations of mercury and arsenic were analyzed in the lungs and brain of control (2 % v/v Tween-80) and treated group of rats after consecutive 28 days pretreatment with different particle sized Shwaskuthar Rasa samples. To determine the accumulation of heavy metals (mercury and arsenic), on 29th day, two rats per group were sacrificed and the organs were removed, weighed, and frozen at -20 °C prior to analysis. Piece of brain and lungs were dried at 60 °C to a constant weight and tissues were dissolved in a mixture of concentrated nitric– perchloric acid 4:1 (Sawicka- Kapusta et al., 1987). Mercury and arsenic were measured by inductively coupled plasma mass spectrometer (ICP-MS, Perkin-Elmer ELAN-6000) and their concentrations were calculated and expressed in µg/g on a dry weight basis.
The safety/toxicity studies of different particle sized *Shwaskuthar Rasa* samples were carried out in albino rats. In single dose oral toxicity/safety studies of in-process withdrawn samples of *Shwaskuthar Rasa*, all group of rats received single dose of samples of *Shwaskuthar Rasa* as 23 mg/kg/day for 28 days. No signs of toxicity and mortality were observed in the animals of treated group as compared to the control group. The data revealed that different particle sized *Shwaskuthar Rasa* samples on the body weight of animals (Table 7.1 and Fig 7.1) no significant differences in weight gain of treated groups compared to control group animals.
Haematological studies

Haematological examination showed that the red blood cells was increased from 7.26 \((10^6/mm^3)\) of SWR 1220 treated group to 7.79, 8.16, 8.74, 9.78 \((10^6/mm^3)\) of SWR 829, SWR, 574, SWR 216, SWR 92 treated group respectively. Statistically red blood cells were significant \((p<0.05)\) increased in SWR 92 treated group compared to control and other withdrawn samples of *Shwaskuthar Rasa* treated groups (*Table 7.2 and Fig 7.2*). Similarly concentration of haemoglobin was increased from 11.82 \((g/dl)\) of SWR 1220 treated group to 12.59, 13.42, 14.85, 16.67 \((g/dl)\) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (*Table 7.2 and Fig 7.3*). Statistically, concentration of haemoglobin showed significant \((p<0.05)\) increase in SWR 92 treated group compared to control and other treated groups. These data clearly indicated that decrease in particle size of *Shwaskuthar Rasa* samples enhanced the RBCs count and concentration of haemoglobin.

Whereas count of white blood cells was decreased from 11.69 \((10^3/mm^3)\) of SWR 1220 treated group to 11.32, 10.96, 10.37, 9.78 \((10^3/mm^3)\) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (*Table 7.2 and Fig 7.4*). Statistically white blood cells were significant \((p<0.05)\) decreased in SWR 92 treated group compared to control and other treated groups. The effect of in-process withdrawn samples of *Shwaskuthar Rasa* on differential white blood cells count (eosinophil, basophil, neutrophil, monocyte and lymphocyte) was studied. The eosinophil cells were decreased from 2.88 % of SWR 1220 treated group to 2.71, 2.52, 2.27, 2.04 % of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (*Table 7.2 and Fig 7.5*). In case of basophil cells, they were decreased from 1.66 % of SWR 92 treated group to 1.58, 1.47, 1.31, 1.02 % of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (*Table 7.2 and Fig 7.6*). In addition, neutrophil cells was decreased from 20.11 % of SWR 1220 treated group to 20.02, 17.30, 16.18, 15.21 % of SWR 829, SWR, 574, SWR 216, SWR
92 treated groups respectively (Table 7.2 and Fig 7.7). As well monocyte cells were decreased from 3.88 % of SWR 1220 treated group to 3.71, 3.44, 3.13, 3.04 % of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.2 and Fig 7.8). Also lymphocyte cells were decreased from 87.32 % to 82.01, 79.34, 75.09, 74.86 % of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.2 and Fig 7.9). Statistically eosinophil and basophil were significantly (p<0.05) decreased in SWR 92 treated groups compared to control and other treated groups with different particle sized Shwaskuthar Rasa. Whereas neutrophil, monocyte and lymphocyte cells notably decreased in SWR 92 groups. All haematological values of RBCs, WBCs and differential WBCs count were found within the normal limits, thus results can be considered as normal. These changes suggest the different particle sized Shwaskuthar Rasa was safe on haematological parameters and that no sign of toxicity were observed/noticed.

**Biochemical studies of liver functions**

The effect of different particle sized Shwaskuthar Rasa samples on liver function parameters were studied. The concentration of serum glutamate oxaloacetate transaminase (SGOT) was showed decrease from 118.24 (U/I) of SWR 1220 treated group to 102.33, 97.08, 94.50, 90.24 (U/I) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.3 and Fig 7.10) with statistically significant (p<0.05) decrease in SWR 216 and SWR 92 groups. Similarly the concentration of serum glutamate pyruvate transaminase (SGPT) showed decrease from 65.45 (U/I) of SWR 1220 treated group to 62.60, 59.18, 55.22, 52.88 (U/I) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.3 and Fig 7.10) with statistically significant (p<0.05) decrease in SWR 92 group compared to other treated group. In addition the concentration of alkaline phosphatase (ALP) was showed decrease from 135.60 (U/I) of SWR 1220 treated group to 116.37,
103.45, 98.08, 95.16 (U/I) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.3 and Fig 7.10) with statistically significant (p<0.05) decrease in SWR 216 and SWR 92 groups compared to other treated groups. Liver function parameter of direct bilirubin was showed decrease from 0.41 (mg/dl) of SWR 1220 treated group to 0.37, 0.32, 0.25, 0.23 (mg/dl) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.3 and Fig 7.11). And statistically significant (p<0.05) decrease of direct bilirubin in SWR 216 and SWR 92 group compared to other Shwaskuthar Rasa samples treated groups was observed. Also concentration of total bilirubin was decreased from 1.65 (mg/dl) of SWR 1220 treated group to 1.52, 1.30, 1.12, 0.96 (mg/dl) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.3 and Fig 7.11). With statistically concentration of total bilirubin was significant (p<0.05) decrease in SWR 216 and SWR 92 treated group compared to other treated groups were found. Liver function parameter data overall revealed that reduction in particle size of Shwaskuthar Rasa did not hamper biochemical parameters of liver.

Biochemical studies of kidney functions

The effect of different sized Shwaskuthar Rasa samples on kidney function biochemical parameters were studied. These findings showed that as particle size of formulation decreases from SWR 1220 nm to SWR 92 nm, the biochemical parameters such as serum urea and serum creatinine level are gradually reduced from 27.12 (mg/dl) to 19.74 (mg/dl) and 1.32 (mg/dl) to 0.76 (mg/dl) respectively (Table 7.4 and Fig 7.12) and finally approached to near normal value. Statistically significant (p<0.05) decrease in serum urea and serum creatinine in SWR 216 and SWR 92 group compared to other treated groups. These data suggest that decrease in particle size of formulation was safe on kidney biochemical function parameter.
Biochemical studies of heart functions

The effect of different sized *Shwaskuthar Rasa* samples on heart function biochemical parameters were studied. These findings showed that as particle size of formulation decreases, the lactate dehydrogenase level was reduced from 276.46 (U/I) of SWR 1220 to 264.08, 253.52, 247.06, 238.12 (U/I) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.5 and Fig 7.13) with statistically significant (p<0.05) reduction of lactate dehydrogenase in SWR 216 and SWR 92 groups compared to other *Shwaskuthar Rasa* treated groups. Similarly particle size reduction of formulation caused decrease in creatine kinase level from 188.77 (U/I) by SWR 1220 to 179.16, 175.18, 169.27, 166.35 of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.5 and Fig 7.13) and statistically significant (p<0.05) decrease in creatine kinase level in SWR 92 group compared to other *Shwaskuthar Rasa* treated groups. Values of lactate dehydrogenase (LDH) and creatine kinase (Ck) finally approached near to normal. Thus the study clearly indicated that reduction in particle size of *Shwaskuthar Rasa* is beneficial as smaller size of formulation did not hamper the biochemical parameters. It also reveals that as particle size of formulation is reduced the particle size of mercury and arsenic present in formulation also decreases and their possible effect on biochemical parameters decreased gradually.

Urine and fecal analysis

Mercury and arsenic was analyzed in urine and faecal samples. In urine analysis (Table 7.6 and Fig 7.14) showed increase in mercury levels from 0.268 (ppm) of SWR 1220 group to 0.312, 0.359, 0.397, 0.424 (ppm) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively with statistically significant (p<0.05) concentration of mercury increased in SWR 216 group and highly significant (p<0.01) mercury increased in SWR 92 group. Similarly detection of arsenic was
increased from 1.516 (ppm) of SWR 1220 to 1.784, 1.967, 2.235, 2.564 of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively with statistically significant (p<0.05) concentration of arsenic increased in SWR 216 and SWR 92 groups compared with other treated groups. But in control group there is no detection of mercury and arsenic content in urine. These studies clearly indicated that reduction of particle size of *Shwaskuthar Rasa* preparation was directly proportional to elimination of mercury and arsenic through urinary routes of rats.

In addition fecal analysis (*Table 7.7 and Fig 7.15*) showed detection of mercury increased from 0.517 (ppm) of SWR 1220 to 0.545, 0.663, 0.692, 0.784 (ppm) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively with statistically significant (p<0.05) mercury increased in SWR 216 and SWR 92 treated groups whereas detection of arsenic gradually increased from 6.641 (ppm) of SWR 92 group to 6.880, 7.615, 7.746, 8.132 of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively and arsenic did not showed statistically difference among treated groups. The data clearly indicated that the excretion of mercury and arsenic through feces depends on the fineness of *Shwaskuthar Rasa* i.e. finer the particle size the more the excretion of mercury and arsenic in urine and feces of rats.

**Organ weight and histology**

The effect of different particle sized *Shwaskuthar Rasa* was studied on organ weight for liver, kidney, heart, brain and lung. The data (*Table 7.8 and Fig 7.16*) revealed that there were no significant difference in organ weight between the control and treated groups. These studies indicated that all the treated group of organ weight is near to normal organ weight. Finally the reduction of particle size of preparation did not hamper the organ weight of all *Shwaskuthar Rasa* treated groups and control group. The study revealed that all the particle sized of preparation of *Shwakuthar Rasa* are safe.
Hematoxylin 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 (Photograph 7.1 A). In contrast, the SWR 1220 and SWR 829 treated group revealed the moderate damage of any of the groups, the liver sections showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries (Photograph 7.1 B and 7.1 C). The liver sections of the rats treated with SWR 574, SWR 216, SWR 92 (Photograph 7.1 D, 7.1 E and 7.1 F) showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration which were almost comparable to the control (Photograph 7.1 A).

The histological examination of the hematoxylin and eosin stained control kidney showed normal architecture of renal tubular epithelial cells and presence of hyaline casts in the tubular lumen (Photograph 7.2 A). The degenerative change was lessened by SWR 1220 treated group, indicating a partial nephroprotective effect (Photograph 7.2 B). 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 was observed (Photograph 7.2 C). Glomeruli did not show any changes. Less damage to renal tubular epithelial cells with inflammatory cells was observed with SWR 574 (Photograph 7.2 D). However, no remarkable effects in the groups treated with SWR 216 and SWR 92 (Photograph 7.2 E and 7.2 F) compared with the control group (Photograph 7.2 A).

The histoarchitecture of cardiac tissues of control group appeared to be normal as there was no visible necrotic damage to the myocytes (Photograph 7.3 A). However, moderate myocyte membrane damage, myonecrosis, fibroblastic proliferation and infiltration of inflammatory
cells were observed in SWR 1220 and SWR 829 treated groups (Photograph 7.3 B and 7.3 C). Scrutiny of cardiac tissues of SWR 574 group (Photograph 7.3 D) revealed that there was minimum damage to the myocardium with much reduced myonecrosis and lymphocyte infiltration than SWR 1220 group. Whereas, SWR 216 and SWR 92 groups (Photograph 7.3 E and 7.3 F) showed a more or less normal damage to the myocardium compared with control group (Photograph 7.3 A) as there was practically no change.

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 contains dendrites and numerous neuroglia (Photograph 7.4 A). Cerebellum section of SWR 1220 showed molecular layer appeared with highly vacuolated cytoplasm showing degenerative changes, decreased in their number or completely disappeared in some areas. Other area showed a progressive degree of febricity and a wavy appearance of nerve fibers (Photograph 7.4 B). Treatment with SWR 829 and SWR 574 showed purkinje cells slightly shrinking 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 (Photograph 7.4 C and 7.4 D). However, SWR 216 and SWR 92 treated groups the cerebellum sections revealed did not remarkable pathological changes observed in purkinje cells, molecular layer, granular layer cells and the white matter compared to among treated groups (Photograph 7.4 E and 7.4 F).

Histological findings of lung tissues of control group appeared to be normal as there was no pulmonary alterations such as inflammatory cellular infiltration, bronchiolar associated lymphoid tissue (BALT)
hyperplasia and perivasculitis (Photograph 7.5 A). However, SWR 1220 and SWR 829 have shown moderate pulmonary alterations such as inflammatory cellular infiltration, BALT hyperplasia and perivasculitis (Photograph 7.5 B and 7.5 C). SWR 574 showed mild tubular injury, BALT hyperplasia, lymphohistiocytic perivasculitis and bronchoalveolar inflammatory infiltrate (Photograph 7.5 D). Whereas, SWR 216 and SWR 92 groups (Photograph 7.5 E and 7.5 F) showed a more or less normal damage to the lungs compared with control group (Photograph 7.5 A).

The study indicated that the different particle sized Shwaskuthar Rasa had no serious toxicity potential to most of the important organs at therapeutic dose levels. Besides, there were no apparent gross lesions at necropsy and histological examination of liver, kidney, heart, brain and lungs did not reveal extreme pathological changes at therapeutic dose. This may be due to the presence of metals or minerals in the formulation as oxide / sulphide/ salt forms which are non-toxic. With our findings which seem to be the safety profile of the SWR 216 and SWR 92 treated group compared with SWR 574, SWR 829 and SWR 1220 group in rat organs. However, chronic safety / toxicity studies would be needed in this area.

**Tissue enzymes of lungs and brain**

Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, 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 (Meistor, 1983).

Administration of different particle sized Shwaskuthar Rasa in rats (23 mg/kg, p.o.) for 28 days resulted in decreased brain tissue lipid peroxidation (LPO) from 25.42 (nmol/mg) by SWR 1220 to 22.86, 19.05,
17.43, 15.89 (nmol/mg) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.9 and Fig 7.17) and statistically significant (p<0.05) decreased level of brain tissue lipid peroxidation in SWR 92 treated group compared to other treated groups and SWR 92 group showed near normal value of lipid peroxidation (LPO) i.e. 16.12 (nmol/mg). Whereas brain tissue glutathione (GSH) showed increase from 0.65 (µmol/mg) of SWR 1220 to 0.88, 1.56, 2.19, 2.80 (µmol/mg) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.9 and Fig 7.18) and statistically significant (p<0.05) brain glutathione level increased in SWR 216 treated group and highly significant (p<0.01) increased in SWR 92 treated group compared with control and other Shwaskuthar Rasa treated groups. It indicated that reduction in particle size of Shwaskuthar Rasa preparation decreased lipid peroxidation and increased the glutathione level in brain tissue. It is important to note that such type of changes in lipid peroxidation and glutathione levels in brain tissues are considered positive.

Similarly administration of different particle sized Shwaskuthar Rasa in rats (23 mg/kg, p.o) for 28 days resulted in decreased lung tissue lipid peroxidation from 21.39 (nmol/mg) by SWR 1220 to 16.15, 15.40, 14.33, 12.48 (nmol/mg) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.10 and Fig 7.19) and statistically significant (p<0.05) decreased lung tissue lipid peroxidation level in SWR 92 treated group compared to other treated group and showed near normal value of lipid peroxidation (LPO) i.e. 12.79 (nmol/mg). Whereas lung tissue glutathione (GSH) showed increase from 0.41 (µmol/mg) of SWR 1220 to 0.79, 1.23, 1.98, 2.35 (µmol/mg) of SWR 829, SWR, 574, SWR 216, SWR 92 treated groups respectively (Table 7.10 and Fig 7.20) with statistically significant (p<0.05) lung glutathione level increased in SWR 574 treated group and highly significant (p<0.01) decreased in SWR 216 and SWR 92 treated groups compared to control and other Shwaskuthar Rasa treated groups.
With these findings, it indicated that *Shwaskuthar Rasa* exhibits tissue protection on through antioxidant action by inhibiting of lipid peroxidation and enhancing glutathione level and, thus used in the treatment of asthma and allergy effects where reactive oxygen species are involved. The study revealed that significant decrease in lipid peroxidation and enhancement glutathione levels in brain and lung tissue of rats based on fineness of formulation suggests that the *Shwaskuthar Rasa* preparation has protective effect against necrotic changes in these body organs.

**Accumulation of heavy metals**

Evaluated the concentrations of so-called heavy metals as mercury and arsenic in brain and lung tissue of rats pretreated with different particle sized *Shwaskuthar Rasa* (23 mg/kg, p.o) repeatedly for 28 days since mercury and arsenic were affecting wide range of physiological functions include nervous, excretory, reproductive, respiratory, and haematopoietic systems, if more than the exceeded limits.

The data findings *(Table 7.11 and Fig 7.21)* showed that as particle size of formulation decreases from 1220 nm to 92 nm, the detection of mercury and arsenic in brain tissues are gradually reduced from 0.134 to 0.082 (ppm) and 0.047 to 0.020 (ppm) respectively. In control group did not detection of mercury and arsenic in brain tissue. Statistically significant (p<0.05) decreased accumulation of mercury and arsenic in brain tissue treated with SWR 216 and SWR 92 group compared to other treated groups. These data suggest that decrease in particle size of *Shwaskuthar Rasa* formulation is directly proportional to accumulation of mercury and arsenic in brain tissues i.e. finer the particle size of formulation lesser would be the accumulation. The outcome of the study therefore is that as accumulation due to finer particle of preparation is very less, the chance of so-called heavy metal
toxicity are reduced to a great extent making the formulation safe for human consumption as therapy for asthma and allergy.

Correspondingly in the lungs, the data (Table 7.11 and 7.21) revealed that as particle size of formulation decreases from 1220 nm to 92 nm, detection of mercury and arsenic decreased from 0.085 to 0.044 (ppm) and 0.021 to 0.011 (ppm) respectively. In control group no mercury and arsenic in lung tissue was detected. Statistically significant (p<0.05) decreased accumulation of mercury and arsenic in lung tissue treated with SWR 216 and SWR 92 group compared to other treated groups. These data suggest that decrease in particle size of Shwaskuthar Rasa formulation is directly proportional to accumulation of mercury and arsenic in lung tissues. The detection of accumulation of mercury and arsenic was found in brain and lung tissues of different particle sized Shwaskuthar Rasa treated rats was within permissible limits. The study finally revealed that mercury and arsenic did not accumulation in brain and lung tissues at dangerous level instead excreted through urine and feces after performed its therapeutic action in cellular level.