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*Original Article*

# Pharmacovigilance for safer use of drugs in Ayurveda through Experimental study

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## ABSTRACT

***Background:*** *Heavy metals are used in Ayurvedic medicine since ancient period. Case reports published in national and international journals and news papers regarding heavy metals poisoning after the use of drugs in Ayurveda has created a negative impact on public towards the use of Ayurvedic medicine. On the ground where formulations of Ayurved are targeted to contain high levels of heavy metals, there is urgent need to have pharmacovigilance for these drugs regarding their safety in the treatment. Hence, an experimental study was conducted to assess the toxicity and to identify the factor responsible for the toxicity of the arsenical compounds Rasamanikya which is a very popular medicine used for various ailments.*

***Methods:*** *Physical and physico-chemical properties of three different market preparations of Rasamanikya were studied by using advance methods (ICP-AES and XRD) and the sample containing highest level of Arsenic was subjected to chronic toxicity study in rats. Hematological and biochemical parameters were assessed at 30, 60 and 90 days and histopathological study was conducted at the end of the study.*

***Result:*** *All the three samples showed variation in concentration of arsenic and its crystal size. Traces of nineteen other metal compounds were found in all samples other than Arsenic and Sulphur. There were no significant differences observed most of hematological and biochemical parameters up to 60 days in Wistar rats. No significant differences in body weight and relative organ weight were observed. Minimal to mild changes were observed in liver and kidney which were pathologically insignificant.*

***Conclusion:*** *LD 50 of Rasamanikya may be more than 2000 mg/kg and Rasamanikya is safe for 15 days duration in the therapeutic dose in human beings. Chronic Arsenic toxicity due to Rasamanikya is not possible if it is consumed in therapeutic dose for 10- 15 days. But there is an urgent need of standardization of method of preparation and standardization of contents to prepare Rasamanikya and its use for therapeutic purposes.*

***Key words:*** *Pharmacovigilance, Heavy metal toxicity, Rasamanikya, Chronic Arsenic toxicity*

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## INTRODUCTION

WHO has defined Pharmacovigilance as the science which deals with the activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem. Along with adverse event reporting, awareness for the safe use of drugs is also mandatory.

In the recent past, many claims have appeared regarding the toxicity of Ayurvedic medicines in various contexts. It may divert the faith of patients and value of Ayurveda. The American medical research community has sounded a heavy metal warning against Ayurvedic cures. They have declared that Ayurvedic medicine users may be at risk for heavy metal toxicity. The articles like **"Poison in Ayurvedic Drugs"** published in Times of India, Ahmadabad on 31/03/2017, **"Ayurvedic med poses life threat"** published in Pune Mirror on 25/12/2017 and **"Ayurvedic herbal drugs damaging liver: Study"** published in Times of India, Kochi edition on 09/03/2018 may lead to distrust in the minds of the readers regarding safety, efficacy and overall creditability of AYUSH systems in general and of Ayurvedic drugs in particular. So it is our duty towards our science that we should reexamine the Ayurvedic Products whether they are free from toxic properties of heavy metals with the help of recent advanced techniques.

Herbs, metals and minerals or in combination with herbs as herbomineral formulations are described in Ayurveda and used for treatment. Minerals like Mercury, Lead, Arsenic, Copper etc are used since ancient period in these formulations. Arsenic poisoning is a medical condition caused by elevated levels of arsenic in the body. Chronic arsenic poisoning is well known and established

only by research in modern toxicology. The various sources for chronic arsenic poisoning are arsenic contaminated water and soil, sea fishes, wall paints and colors, hair removing creams, cosmetics, crayons (chalk), fireworks, fungicides, insecticides, pesticides, weed killers, rat poison, sheep dip, printer's ink, depilatory, toys, candles, fabric, wallpaper, household lawn and garden chemicals, arsenic-treated wood etc. *Rasamanikya* also contains arsenic compounds, therefore it is also considered as a source of chronic arsenic poisoning. As *Rasamanikya* is widely used in South India, North-east India and by various vaidyas due to its more therapeutic uses, chronic poisoning may occur due to its long term use and or without prescription or it is consumed in large doses. So to avoid acute, sub-acute and chronic toxicity of *Rasamanikya*, it is necessary to limit the doses and duration of administration. Hence, this research topic was selected for detail study of chronic arsenic poisoning supposed to occur from the long term use of *Rasamanikya* under heavy metal toxicity

## METHODS

Approval from Animal Ethical Committee was received from APT Research Foundation for Research Project No "RP. No. 35/1718" dated 08/August/2017. Market sample of *Rasamanikya* 100 gm was procured from local market. The details mentioned on container of the sample like reference of formulation, batch number, date of manufacturing, date of expiry and precautions were noted. XRD and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was conducted at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Bombay. The chronic toxicity study was conducted at APT Research Foundation (National Toxicology Centre), Pune.

**Estimation the LD50 of *Rasamanikya*:**

To estimate the LD50 of *Rasamanikya,* acute oral toxicity study was conducted by following OECD Guideline 423 (Acute toxic class method) at a limit dose of 2000 mg/kg of *Rasamanikya* orally.

**Chronic oral toxicity:**

To evaluate the chronic toxicity of *Rasamanikya*, repeated dose chronic oral toxicity study was conducted in Wistar Albino rats for 90 days by following OECD Guideline 408.

**Dose selection and preparation:**

Dose of the drug was calculated by extrapolating the human therapeutic dose to rats on the basis of body surface area ratio (conversion factor 0.018 for rat). The selected dose of *Rasamanikya* was 250 mg by weight for Human.

Absolute Dose for 200 gm rat =Human dose x Conversion factor for rat = 250 x 0.018 = 4.5 mg

Rat dose /kg = 22.50 mg/kg

Therefore, Therapeutic exposure dose (TED)- 22.50 mg/kg; 5 times TED- 112.5 mg/kg and 10TED- 225 mg/kg of *Rasamanikya* was given for 90 days orally with honey as vehicle.

**Table No. 1: Dose schedule of *Rasamanikya* (RM):**

| Group | Pattern of dosing | Dose of *Rasamanikya* given to Rats |
|---|---|---|
| Group A VC | Vehicle Control Without *Rasamanikya* | - |
| Group B RM TED | *Rasamanikya* in therapeutic exposure dose (TED) | 22.50 mg/kg |
| Group C RM 5TED | *Rasamanikya* in increasing dose (5 times TED) | 112.5 mg/kg |
| Group D RM 10TED | *Rasamanikya* in increasing dose (10 times TED) | 225 mg/kg |

**Administration of test article:** Fine suspension of *Rasamanikya* was prepared in honey (vehicle) and distilled water as per dose level and it was administered between 10:00 am to 10:30 am daily for 90 consecutive days to each rat by a single oral gavage. The animals were dosed using a stainless steel intubation needle fitted onto a suitably graduated syringe. The dosage volume administered to individual rat was adjusted according to its most recently recorded body weight.

**Assessment:**

Body weight was assessed before the commencement of dosing followed by weekly once during the dosing period and once on the terminal day. Food and water consumption were measured weekly. During study, the various hematological (WBC, RBC, Hemoglobin, Hematocrit and Platelet count) and biochemical parameters (Total Bilirubin, SGPT, Alkaline phosphatase, total protein, glucose, urea, creatinine, sodium, potassium and cholesterol) were analyzed after every 30 days. At the end of study, hematological and biochemical parameters were analyzed. Urine analysis was also performed at 30, 60 and 90 days. Blood was collected by supra-orbital puncture with the help of micro capillary tubes. The weights of the organs were recorded (absolute organ weight) and Relative organ weight was calculated as follows:

Relative organ weight (gm) = absolute organ weight (gm)/ terminal body weight (gm)

The organs were preserved soon after dissection in 10% formalin solution for fixation for histopathological examination. Brain, heart, lungs, stomach, intestine, liver, spleen, kidney, uterus, ovaries and testes were subjected to histopathological study. The sections of different organs from treated group were compared with the sections of normal vehicle control group.

**Statistical analysis:**

The results are presented as Mean± Standard Error (SE) of means in each group. Statistical comparisons were performed by both paired, unpaired student's t test by using Sigma stat software (version 3.1) for all the experimental groups. Unpaired t test was applied between the studied groups as well as paired t test was applied within the individual group at 30, 60 and 90 days. All the parameters were subjected to One Way ANOVA test followed by Multiple Comparison Procedures (Holm-Sidak method) to determine the significant difference between the groups at $P<0.05$ (level of significance).

## RESULTS

**Table No. 2: Showing observations of physicochemical analysis.**

| Sr. No. | Analytical Parameter | Sample A | Sample B | Sample C |
|---|---|---|---|---|
| 1 | Loss on Drying at $105^0$ C | 0.16% | 1.66% | 0.4% |
| 2 | Total Ash Value | 1.1% | 1.76% | 1.1% |
| 3 | Acid insoluble Ash value | 0.53% | 1.06% | 0.56% |
| 4 | Water soluble extractive value | 37% | 30% | 39% |
| 5 | Alcohol soluble extractive value | 6% | 4% | 7% |
| 6 | pH | 6.03 | 6.00 | 6.09 |
| 7 | Particle Size (By Sieve Analysis using 100 No. Mesh having standard 0.149mm) | 98.5% | 98% | 97% |
| 8 | ICP-AES for Arsenic (As) | 58.29 % | 64.42 % | 67.18 % |
| 9 | XRD Analysis | Average Crystal Size | 110.308 nm | 167.884 nm |
| | | Average Lattice Strain | 0.0028 | 0.0046 |

**Table No. 3: Showing observations of Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES).**

| Sample | Qualitative Analysis Report (presence of elements) | Number of elements detected | Quantitative Analysis Report / Arsenic (As) estimation:- % |
|---|---|---|---|
| Sample A | Ag, Al, As, B, Ba, Ca, Cl, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, S, Sb, Si, Sr, Ti, Zn | 21 | 58.29 |
| Sample B | Ag, Al, As, B, Ba, Ca, Cl, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, S, Sb, Si, Sr, Ti, Zn | 21 | 64.42 |
| Sample C | Ag, Al, As, B, Ba, Ca, Cl, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, S, Sb, Si, Sr, Ti, Zn | 21 | 67.18 |

**Estimation of LD50:**

Acute oral toxicity of *Rasamanikya* performed at a limit dose of 2000 mg/kg by OECD 423 was found to be safe. No mortality was observed during the course of study (14 days). Gross behavior of all the animals was found to be normal during the period of study. From this, it can be inferred that the LD50 value of *Rasamanikya* is higher than 2000 mg/kg body weight in the rats.

In chronic toxicity study, no behavioral changes were found in all the experimental groups during 90 days and no mortality was observed in any of the groups at TED, 5 TED and 10 TED level. Fecal and urine output remained unaffected in all the four groups. There was no significant change in body weight during the 90 days of study in all experimental groups in comparison with control group.

**Table No 4: Effect of *Rasamanikya* on body weight.**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| At 28 days | 242.833±7.346 | 236.000±8.331 | 235.667±9.073 | 230.667±11.806 |
| At 63 days | 280.500±6.927 | 271.500±10.016 | 270.667±10.324 | 260.333±12.593 |
| At 91 days | 304.667±7.843 | 295.333±10.161 | 294.333±10.667 | 283.500±13.740 |

*Significant, **Highly Significant

**Table No 5: Effect of *Rasamanikya* on hematological parameters at 30 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| WBC | 6.667±0.749 | 6.233±0.745 | 8.550±0.583 | 8.300±0.838 |
| RBC | 8.217±0.169 | 8.467±0.411 | 8.172±0.217 | 8.158±0.278 |
| HB | 15.700±0.409 | 16.033±0.771 | 15.817±0.349 | 15.400±0.514 |
| HCT | 62.200±1.377 | 63.500±2.699 | 63.067±1.464 | 61.417±1.761 |
| PLT | 543.500±39.824 | 578.333±57.571 | 684.500±50.625 | 610.500±29.492 |

*Significant, **Highly Significant

**Table No 6: Effect of *Rasamanikya* on hematological parameters at 60 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| WBC | 12.133±0.363 | 8.717±0.644* | 8.967±0.645* | 9.767±1.589 |
| RBC | 8.255±0.207 | 8.357±0.259 | 7.843±0.103 | 7.918±0.124 |
| HB | 14.983±0.409 | 14.850±0.313 | 14.267±0.259 | 14.200±0.214 |
| HCT | 62.800±1.592 | 62.367±1.483 | 60.033±0.986 | 60.250±0.889 |
| PLT | 619.833±24.478 | 653.833±37.080 | 714.333±23.606* | 668.167±17.327 |

*Significant, **Highly Significant

**Table No 7: Effect of *Rasamanikya* on hematological parameters at 90 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| WBC | 9.900±0.724 | 6.333±0.417* | 5.883±0.767* | 5.750±0.680* |
| RBC | 0.680±0.230 | 7.680±0.249 | 7.587±0.110 | 7.448±0.258 |
| HB | 14.883±0.334 | 13.817±0.353 | 13.817±0.201 | 13.283±0.221* |
| HCT | 60.967±1.480 | 56.783±1.461 | 57.483±0.607 | 55.683±1.044 |
| PLT | 601.833±31.974 | 585.000±29.290 | 629.000±16.711 | 580.000±9.873 |

*Significant, **Highly Significant

**Table No 8: Effect of *Rasamanikya* on biochemical parameters at 30 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| Total Bilirubin | 0.747±0.126 | 1.877±0.671 | 1.680±0.618 | 1.188±0.384 |
| SGPT | 50.570±4.139 | 40.297±3.226 | 37.913±2.395 | 42.123±4.780 |
| ALP | 105.333±7.974 | 106.667±10.996 | 89.667±4.595 | 93.833±7.888 |
| Protein | 6.283±0.340 | 6.350±0.178 | 6.250±0.305 | 6.517±0.125 |
| Glucose | 85.167±4.238 | 88.000±2.422 | 88.500±3.442 | 87.167±3.701 |
| Urea | 33.108±2.885 | 34.365±3.054 | 39.393±3.641 | 32.295±1.233 |
| Creatinine | 0.483±0.0872 | 0.367±0.0422 | 0.400±0.0258 | 0.400±0.0365 |
| Sodium | 140.313±4.653 | 146.097±1.958 | 145.835±5.179 | 150.782±8.603 |
| Potassium | 5.298±0.407 | 4.838±0.287 | 5.222±0.236 | 5.918±0.283 |
| Cholesterol | 47.667±1.783 | 49.833±1.956 | 60.167±2.455* | 53.500±1.232* |

*Significant, **Highly Significant

**Table No 9: Effect of *Rasamanikya* on biochemical parameters at 60 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| Total Bilirubin | 0.450±0.0671 | 0.983±0.328 | 0.900±0.314 | 0.650±0.198 |
| SGPT | 52.950±2.980 | 43.067±1.886* | 42.417±1.773* | 40.183±3.223* |
| ALP | 116.500±5.960 | 114.583±7.998 | 109.417±4.777 | 108.833±5.429 |
| Protein | 6.733±0.186 | 6.833±0.278 | 6.367±0.372 | 6.683±0.241 |
| Glucose | | | | |
| Urea | | | | |
| Creatinine | | | | |
| Sodium | | | | |

| | | | | |
|---|---|---|---|---|
| Potassium | 88.167±5. | 89.833±2. | 87.833±3. | 86.167±2. |
| Cholesterol | 023 | 561 | 219 | 386 |
| | 37.850±1. | 36.450±2. | 42.750±2. | 35.267±1. |
| | 497 | 478 | 749 | 493 |
| | 0.783±0.1 | 0.517±0.0 | 0.617±0.0 | 0.617±0.0 |
| | 92 | 703 | 307 | 477 |
| | 150.333± | 136.667±1 | 148.333± | 141.000± |
| | 0.989 | 0.207 | 2.777 | 3.077 |
| | 8.467±0.4 | 7.817±0.4 | 7.600±0.8 | 7.800±0.2 |
| | 35 | 53 | 34 | 99 |
| | 46.000±1. | 41.500±3. | 41.333±1. | 40.333±0. |
| | 633 | 263 | 116* | 843 |

*Significant, **Highly Significant

**Table No 10: Effect of *Rasamanikya* on biochemical parameters at 90 days**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| Total | 0.1000±0. | 0.1000±0. | 0.1000±0. | 0.1000±0. |
| Bilirubin | 000 | 0258 | 000 | 000 |
| SGPT | 55.350±2. | 45.867±1. | 46.950±2. | 38.250±2. |
| ALP | 458 | 136* | 039* | 545* |
| Protein | 119.500± | 124.833±7 | 127.167± | 124.333± |
| Glucose | 7.356 | .213 | 8.420 | 9.549 |
| Urea | 7.900±0.2 | 8.050±0.3 | 8.200±0.5 | 8.083±0.3 |
| Creatinine | 82 | 17 | 22 | 59 |
| Sodium | 97.500±3. | 110.500±1 | 95.000±5. | 85.333±6. |
| Potassium | 981 | .893* | 972 | 458* |
| Cholesterol | 42.567±1. | 38.517±2. | 46.117±2. | 38.250±2. |
| | 715 | 115 | 244 | 545 |
| | 0.450±0.0 | 0.483±0.0 | 0.350±0.0 | 0.517±0.0 |
| | 563 | 601 | 563 | 601 |
| | 145.167± | 146.000±3 | 138.167± | 148.500± |
| | 2.330 | .376 | 3.628 | 2.405 |
| | 6.767±0.2 | 6.417±0.3 | 5.683±0.1 | 6.750±0.3 |
| | 76 | 17 | 78* | 13 |
| | 61.917±3. | 65.167±5. | 67.917±1. | 66.500±5. |
| | 134 | 138 | 895 | 885 |

*Significant, **Highly Significant

**Table No 11: Effect of *Rasamanikya* on relative organ weight**

| Name of Parameter | Group A VC | Group B RM TED | Group C RM 5TED | Group D RM 10 TED |
|---|---|---|---|---|
| Brain | 0.480±0. | 0.463±0. | 0.486±0. | 0.479±0. |
| Heart | 0129 | 0255 | 0207 | 0314 |
| Lungs | 0.323±0. | 0.285±0. | 0.273±0. | 0.321±0. |
| Liver | 0137 | 0203 | 00819* | 0356 |
| Pancreas | 0.583±0. | 0.530±0. | 0.491±0. | 0.644±0. |
| Spleen | 0305 | 0429 | 0115* | 0409* |
| Kidneys | 3.550±0. | 2.870±0. | 3.055±0. | 3.009±0. |
| Adrenals | 152 | 272 | 158* | 159* |
| Gonads | 0.234±0. | 0.206±0. | 0.227±0. | 0.227±0. |
| | 0309 | 0265 | 0202 | 0285 |
| | 0.254±0. | 0.228±0. | 0.270±0. | 0.278±0. |
| | 0199 | 0242 | 0126 | 0231 |
| | 0.660±0. | 0.599±0. | 0.612±0. | 0.658±0. |
| | 0632 | 0565 | 0347 | 0373 |
| | 0.0302±0 | 0.0295±0 | 0.0298±0 | 0.0357±0 |
| | .00395 | .00295 | .00206 | .00590 |
| | 0.506±0. | 0.526±0. | 0.551±0. | 0.517±0. |
| | 145 | 150 | 127 | 127 |

*Significant, **Highly Significant

**Effect of *Rasamanikya* on cytoarchitecture of different organs in chronic toxicity study:**

There was no toxic effect of *Rasamanikya* on cytoarchitecture of brain, heart, lungs, stomach, intestine, spleen, testes, uterus and ovaries. However, vascular changes were found in hepatic parenchyma of all the rats of group B treated with therapeutic dose and male rats of group C and D treated with five times and ten times therapeutic dose of *Rasamanikya* respectively. Minimal degenerative and necrotic changes of hepatocytes were found in male rats of group B and D where as mild degenerative and necrotic changes of hepatocytes were found in female rats of group B and male rats of group C. Minimal inflammatory changes in hepatic tissue and fatty changes were found in female rats of group B.

Minimal vascular changes were found in kidneys in all the rats of group B and D and female rats of group C. Minimal degenerative and necrotic changes of renal tubules and glomerular changes were found in male rats of group B and D.

## DISCUSSION

In qualitative analysis of all the three samples of marketed *Rasamanikya*, 21 elements were found in each sample. Quantitative analysis suggests that sample C (67.18%) contains more arsenic in comparison to the sample A (58.29%) and sample B (64.42%). The therapeutic dose of *Rasamanikya* described in Ayurvedic texts is 1-2 *Ratti* (125-250mg) twice a day. Hence patient daily consumes 250 mg of *Rasamanikya.* According to the report of arsenic content in three samples, 72 mg to 145 mg of metallic arsenic in sample A, 80 mg to 161 mg of metallic arsenic in sample B and 83 mg to 167 mg of metallic arsenic in sample C enters in stomach after consuming *Rasamanikya* in one day as a therapeutic drug. This Arsenic content is calculated from the percentage of Arsenic found in the entire three sample and its routine therapeutic dose ranging from 125 -250 mg. [Arsenic entering in stomach= Therapeutic dose x percentage of Arsenic content found in a sample / 100].

**Effect on Hematological parameters:**

There was no significant difference observed in haematological parameters at the interval of 30 days and 60 days after administration of *Rasamanikya* to wistar rats when compared among all experimental groups. But significant decrease in white blood cell, haemoglobin, haematocrit value and platelet count was observed only at 90 days interval which was pathologically insignificant. This indicates that *Rasamanikya* has no toxic effect on haematological parameters up to 60 days after administration of *Rasamanikya* with three fold dose levels in Wistar rats. Hence it can be inferred from these results that *Rasamanikya* may not produce toxic effect on haematological parameters with TED, 5TED and 10 TED up to 15 days in human beings.

**Effect on serum biochemical parameters:**

There was no significant difference observed in biochemical parameters at the interval of 30 days and 60 days after administration of *Rasamanikya* to Wistar rats except SGPT and Cholesterol. SGPT was decreased statistically at the interval of 60 days and 90 days when compared among the experimental groups. But the other parameters like bilirubin, alkaline phosphatase, total protein, urea, creatinine and sodium were having no significant difference. Hence the difference observed was pathologically insignificant. This indicates that *Rasamanikya* has no toxic effect on biochemical parameters up to 60 days after administration of *Rasamanikya* in Wistar rats. From these results, it can be said that *Rasamanikya* may not produce toxic effect on biochemical parameters with TED, 5TED and 10 TED up to 15 days in human beings.

**Effect on relative organ weight:**

Relative organ weight can be the most sensitive indicator of an effect of drug toxicity, as significant differences in organ weight between treated and control animals may occur in the absence of any morphological changes. Relative organ weights of all the studied organs are not significant except that of lungs in which it is statistically significant but pathologically insignificant when compared among all experimental groups.

**Effect on cytoarchitecture of organs:**

In arsenic poisoning brain, heart, lungs, stomach, intestines, liver, kidneys, skin and nerves are the targets for toxicity. In the present study, minimal to mild changes in the cytoarchitecture of liver and kidney were observed which are pathologically insignificant. However, the biochemical parameters of liver and kidney are not significantly increased in all the experimental groups. This indicates that the changes occurred in the organs are mild and not severe to cause impaired liver and kidney functions. These parameters are increased in the severe impairment of these organs.

In most of the hematological and biochemical parameters, there were no significant changes up to 60 days in the rats. It indicates that *Rasamanikya* is safe for 15 days duration in the therapeutic dose in human beings.

To rule out heavy metal toxicity due to mineral containing formulations in Ayurved was the main concern in the present study. Hence, *Rasamanikya,* a formulation of *Haratala* (Arsenic trisulphide) was selected to evaluate its safety in Wistar rats. Arsenic toxicity due to *Rasamanikya* is not possible, if it is consumed in therapeutic dose for the short duration i.e. 10- 15 days. This study supports the claim of AYUSH Dept.G.O.I.Vide circular No Z.25023/09/2017 - D CC ( ayush ) Dated: 4- JAN 2018.

## CONCLUSION

Acute oral toxicity of *Rasamanikya* performed to estimate LD 50 at a limit dose of 2000 mg/kg by OECD guideline 423 was found to be safe. LD50 value may be higher than 2000 mg/kg body weight for *Rasamanikya* in the rats and human beings. In the chronic toxicity study, *Rasamanikya* has no statistically and pathologically significant effect on body weight, most of the hematological and biochemical parameters and relative organ weight of the rats.

Minimal to mild changes observed in histopathological examination of liver and kidney were pathologically insignificant as these changes are not observed in all experimental groups with higher dose level as well as they are not supported by their significant elevated biochemical parameters. In most of the hematological and biochemical parameters, there were no significant changes up to 60 days in the rats. It indicates that *Rasamanikya* is safe for 15 days duration in the therapeutic dose in human beings. Hence, if *Rasamanikya* is needed to use for longer period, it may be repeated after a washout period of 15 days in the human beings as a preventive or safety measure. Chronic Arsenic toxicity due to *Rasamanikya* is not possible if it is consumed in therapeutic dose for the short duration i.e. 10- 15 days.

Such type of Pharmacovigilance is needed for the drugs in Ayurveda to prove their safety.

## REFERENCES


1. Department of AYUSH, Govt of India, Ref. No. Z.25023/09/2017-DCC (AUSH) dated: 15, January 2018.
2. Duffus J H. Heavy metals- a meaningless term? Pure Appl. Chem. 74(5). 2003, 793–807.
3. Pillay VV, Modern medical toxicology. Section 3/9, 4th edition, Jaypee brother's medical publishers Pvt. Ltd, New Delhi; 2013. 79- 83.
4. Lawrence Wilson, Toxic metals and detoxification © May 2018, LD Wilson, Consultants, Inc
5. Mishra S N, editor, Hindi commentary Siddhaprada on Rasendra Chintamani of Dhundhukanath, Kushtharogadhikar, 9/128-133, Chaukhamba Orientalia, Varanasi; 2006; 376.
6. Bhatt Krishnaram, Siddha Bheshaja Manimala. Edited with *'Vaishwanara'* Hindi commentary by Kaladhar Bhatt, *Jwara Chikitsitam* 4/66-67, 4th Edition, Chaukhamba Krishnadas Academy, Varanasi; 159–160.
7. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf
8. https://www.oecd.org/chemicalsafety/testing/Revision-OECD-TG408-repeated-dose-90-day-oral-toxicity-study-in-rodents.pdf
9. Dhundhuknath, Rasendra Chinatmani with Siddhaprada hindi translation by Sinddhinandhan Mishra, Kushtharogadhikar 9/128-133, Choukhambha Orientalia, Varanasi; 2006. 376-377.
10. Govinddas, Bhaishajya Ratnawali edited and enlarged by Bramhashankar Mishra with 'Vidyotini'hindi commentary analysis by Ambikadatta Shastri, editor Rajeshwardatta Shastri, Kushtharoga, 54/117-123, 19th edition, Choukhambha Prakashan, Varanasi; 2008. 895-896.
11. Gopinath, Bharat Bhaishajya Ratnakar edited by Shah Nagindas Chhaganlal, Fourth Part, Rasa Prakarana, yoga number 5598, B. Jain Publishers Pvt. Ltd New Delhi. 229.