Evaluation of chronic toxicological profile of herbo-mineral formulations: Shwaskas Chintamani Rasa and its marketed formulation namely Kas Shwas Hari Rasa

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ABSTRACT

Background: Shwaskas Chintamani Rasa (SKC) and Kas Shwas Hari Rasa (KSH) are the Ayurvedic herbo-mineral formulations. These Ayurvedic formulations contain heavy metals which is the reason of concern and might bring up the safety issue.

Objective: This research article is aimed to study chronic toxicity of SKC and KSH for safety aspect in Wistar rats.

Material and method: A study group of 220 healthy rats were divided into six groups. These rats were administered with SKC and KSH formulations where both the formulations were administered for 180 consecutive days. SKC was administered at doses of 58 mg/kg (equivalent to therapeutic dose i.e. TD), 145 mg/kg (2.5 TD), 290 mg/kg (5 TD) and KSH was administered at dose of 58 mg/kg (TD). According to OECD guideline 452, the effect of these formulations was examined on hematology, serum biochemistry and histopathology of various organs.

Results: Both the formulations did not produce any signs or symptoms of treatment related toxicity in both male and female Wistar rats at therapeutic dose (TD), 2.5 times TD and 5 times TD.

Conclusion: Based on these findings, the NOAEL (No observed adverse effect level) for test formulations SKC and KSH tablets in male and female wistar rats concluded to be preclinically safe.

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1. Introduction

Ayurveda is the most ancient medicine system in India and accepted worldwide. It has the old age history since the 2nd Century BC [1]. The literal meaning of Ayurveda is 'science of life'. Ayurvedic medicine system has shown its impact on other traditional methods of curing disease like Tibetan, Chinese and Greek medicine have been impacted by Ayurvedic medicine [2–5]. Almost 80% of Indian subcontinental population is known to use Ayurvedic and herbal formulations to treat their primary health care needs. Ayurveda plays an important role in an individual’s health by maintaining perfect equilibrium between mind and spirit with nature [6–8].

Shwaskas Chintamani Rasa is well known herbo-mineral medicine. It plays an important role in improving strength and immunity. SKC is a drug of choice for respiratory problems like asthma and bronchitis [9]. SKC formulation contains Suvarna Bhasma (calcinated gold), Shodhit Parad (purified mercury), Shodhit Gandhak (purified sulfur), Suvarnamakshik Bhasma (Chalcopyrite), Abhraka Bhasma (calcinated mica), Loha Bhasma (calcinated iron), and Mouktik Bhasma (calcinated pearl). All these ingredients were processed in Kantakari Swarasa (Solanum surattense), Godugdha (goat milk), Yashimadhu Kwath (Glycyrrhiza glabra), and Nagvalli Patra Swarasa (extract of Piper bettle) [10]. Tablets available in market are of 140–160 mg and prescribed as two tablets once or twice day before or after meal or as directed by Ayurvedic physicians [11]. These tablets are generally advised to be taken with Anupanas such as pepper and honey [12].

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Kas Shwas Hari Rasa (KSH) tablets are also frequently prescribed by an ayurvedic practitioners for treatment of respiratory diseases. KSH is the patented drug manufactured by Shree Dhootapapeshwar Limited, Panvel. Each tablet of KSH contains Shwaskas Chintamani Rasa as one of its constituents, Laxmivilas (Naradeeya), Soota-shekhar Rasa and Taleesadi Choorna. All these ingredients were processed in Vasa Kwath (Adhatoda vasica Linn.). These tablets are also advised to be taken with honey, Cow milk, Cow Ghee or Luke warm water.

SKC is the key component of KSH formulations and it is known to contain traces of heavy metals such as mercury, sulfur, etc. Some of these heavy metals are reported to cause toxicity in prolonged usage. This study examines the chronic toxicity of SKC and KSH in rat as animal model in consideration with OECD 452 guidelines for validating its safety [13].

### 3. Materials and methods

#### 2.1. Animals

The experimental protocol was approved from Institutional Animal Ethics Committee of SDARF (IAEC protocol no. SDARF/CT/2019/02/R1). The experiment was conducted as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and according to OECD guideline 452. A total of 220 healthy male and female Wistar rats of age 6–8 weeks were taken from in-house breeding Animal House Facility of Shree Dhootapapeshwar Ayurvedic Research Foundation (SDARF). The body weight of each animal was in between 120 to 180 g. The animals were provided with standard diet and water ad libitum and housed in plastic cages as per standard conditions (temperature 20±2°C, humidity 30–70% and 12 h dark/light cycle). The animals were acclimatized for a minimum period of seven days prior to the experiment. The experimental protocol was approved from Institutional Animal Ethics Committee of SDARF (IAEC protocol no. SDARF/CT/2019/02/R1). The experiment was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

#### 2.2. Chemicals

Shwaskas Chintamani Rasa (SKC) and Kas-shwas hari rasa (KSH) tablets were procured from Shree Dhootapapeshwar Ltd. Panvel. Carboxy Methyl Cellulose (CMC) was procured from Loba chemie Pvt. Ltd., Mumbai, Isoflurane USP was procured from Raman and Weil Pvt. Ltd, Mumbai.

#### 2.3. Experimental design

Rats were randomly assigned into six groups as described in Table 1:

The doses for rat were calculated from human dose by using conversion factor of 0.018 [14]. The therapeutic dose of Shwaskas Chintamani Rasa is two tablets once or twice a day in human (one tablets contain 160 mg; So two tablets represents 320 mg and four tablets represents 640 mg of formulation). The doses were calculated depends upon the body weight of rats (mg/kg) and represented in Table 1.

### Table 1

| Group No. | Groups Dose (mg/kg) | No. of Males | No. of Females |
|-----------|---------------------|--------------|---------------|
| I         | Normal Control 1% CMC | 20           | 20            |
| II        | Low dose group (TD) SKC | 58           | 20            | 20            |
| III       | Mid dose group (2.5TD) SKC | 145          | 20            | 20            |
| IV        | High dose group (STD) SKC | 290          | 20            | 20            |
| V         | Satellite group (STD) SKC | 290          | 10            | 10            |
| VI        | Marketed formulation (TD) KSH | 58           | 20            | 20            |

TD: Therapeutic dose, 2.5 TD: 2.5 times Therapeutic dose, 5 TD: 5 times Therapeutic dose.

### Table 2

| Groups | Mean body weight (g) ± Std. deviation on 1st week | Mean body weight (g) ± Std. deviation on 27th week |
|--------|--------------------------------------------------|--------------------------------------------------|
| Normal Control | 108.1 ± 12.57                                    | 428.9 ± 35.63                                    |
| Low dose (1 TD) | 111.7 ± 11.06                                    | 432.9 ± 22.50                                    |
| Mid dose (2.5 TD) | 114.8 ± 9.36                                     | 440.2 ± 24.16                                    |
| High dose (5 TD) | 105.5 ± 4.03                                     | 447.2 ± 34.57                                    |
| Satellite (5 TD) | 104.1 ± 6.37                                     | 435.9 ± 36.57                                    |
| Marketed formulation (1 TD) | 107.2 ± 11.98                                  | 424.6 ± 19.65                                    |

(A) Body weight (Male)

| Groups | Mean body weight (g) ± Std. deviation on 1st week | Mean body weight (g) ± Std. deviation on 27th week |
|--------|--------------------------------------------------|--------------------------------------------------|
| Normal Control | 109.7 ± 8.56                                    | 287 ± 18.17                                      |
| Low dose (1 TD) | 111.6 ± 7.88                                    | 292.6 ± 13.39                                    |
| Mid dose (2.5 TD) | 107 ± 4.68                                      | 279.2 ± 9.60                                    |
| High dose (5 TD) | 107.2 ± 3.82                                     | 291.7 ± 9.49                                    |
| Satellite (5 TD) | 107.6 ± 5.85                                     | 290.2 ± 11.53                                    |
| Marketed formulation (1 TD) | 104.6 ± 5.79                                  | 288.4 ± 12.60                                    |

(B) Body weight (Female)

Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett’s multiple test for comparison.
then weighed and mixed with Carboxy methyl cellulose (1% w/v); quantity of powder is taken as per Table 1. The freshly prepared immiscible suspension was administered orally to animals once a day for a period of 180 days. Animals from control group were treated with vehicle alone throughout the treatment period. The dose volume administered to each animal was calculated based on a constant factor of 10 ml/kg body weight.

2.5. Chronic toxicity study

The experimental protocol was designed according to the Organization for Economic Cooperation and Development (OECD) guideline 452. All animals were treated for 180 days. The dosage were given to all group animals as per Table 1. The satellite group was kept for 4 weeks after treatment (completion of 180 days) [13].

2.6. Physiological observations

All the animals were keenly observed three times i.e., pre-dosing, during dosing and post-dosing for the following physiological observations:

- General appearance, body posture and position, autonomic nervous system function, motor coordination, reaction during physical handling, environmental stimulation, tremor, convolution, vocalization, aggression, lacrimation, salivation and gait pattern.

2.7. Mortality and physiological signs

All the animals were monitored twice daily throughout the study period for the presence of moribund or mortality. For individual animal, both the physiological examinations (as described in point number 2.6) and physical examinations (once in week) were carried out.

2.8. Body weight

Individual body weight of animal was monitored before experimental dose administration and thereafter weekly till the end of the experiment. At the end of the experiment, overnight fasted body weight was recorded before necropsy.

2.9. Biochemical analysis

All the animals were kept for overnight fasting after completion of 90 days and 180 days of treatment. Animals were anaesthetized with one to two ml of isoflurane before blood collection.

Blood was collected through retro-orbital plexus in two separate vacutainers. For hematological analysis EDTA was used as an anticoagulant. For serum biochemical analysis blood was collected in plain vacutainer.

Table 3a

| Parameters | Group I (Normal Control) | Group II (SKC TD) | Group III (SKC 2.5 TD) | Group IV (SKC 5 TD) | Group V (SKC 5 TD) | Group VI (Marketed formulation) |
|------------|--------------------------|-------------------|------------------------|---------------------|-------------------|----------------------------------|
| WBC (cells/cmm) | 10.84 ± 1.26 | 10.08 ± 1.06 | 10.08 ± 1.13 | 10.18 ± 1.13 | 10.26 ± 1.06 | 10.22 ± 0.90 |
| RBC (million/mm³) | 7.975 ± 0.56 | 7.67 ± 0.67 | 7.557 ± 0.65 | 8.046 ± 0.28 | 7.836 ± 0.63 | 7.994 ± 0.20 |
| Hct (%) | 45.61 ± 2.07 | 44.29 ± 3.33 | 43.14 ± 3.02 | 46.22 ± 1.77 | 44.94 ± 3.79 | 45.22 ± 1.77 |
| MCV (fL) | 57.33 ± 3.29 | 57.81 ± 1.77 | 57.22 ± 3.22 | 57.47 ± 2.34 | 57.44 ± 2.35 | 56.56 ± 1.61 |
| MCH (pg) | 18.60 ± 1.58 | 18.39 ± 2.32 | 19.18 ± 1.63 | 18.19 ± 0.93 | 18.60 ± 1.21 | 18.50 ± 1.21 |
| MCHC (g/dL) | 32.59 ± 0.08 | 32.81 ± 1.65 | 33.51 ± 2.40 | 31.66 ± 0.43 | 32.36 ± 1.61 | 32.74 ± 2.88 |
| Neutrophil (%) | 17.50 ± 3.27 | 18.10 ± 2.47 | 16.90 ± 4.14 | 17.50 ± 2.32 | 18.00 ± 6.12 | 17.80 ± 2.95 |
| Lymphocyte (%) | 78.70 ± 2.86 | 79.20 ± 2.82 | 80.60 ± 3.86 | 80.00 ± 2.53 | 78.60 ± 6.87 | 78.40 ± 2.70 |
| Monocyte (%) | 2.400 ± 1.17 | 1.500 ± 0.97 | 1.200 ± 0.63 | 1.100 ± 0.56 | 1.100 ± 1.64 | 1.400 ± 0.89 |
| Eosinophil (%) | 78.70 ± 2.86 | 79.20 ± 2.82 | 80.60 ± 3.86 | 80.00 ± 2.53 | 78.60 ± 6.87 | 78.40 ± 2.70 |
| Basophil (%) | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Table 3b

Effect of oral administration of Shwaskas Chintamani Rasa and Kas-shwas Hari Rasa tablet on hematology parameters in female after 90 days. Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett’s multiple test for comparison.
Rats from satellite group were overnight fasted after 4 weeks of completion of treatment and blood was collected. The further procedure was repeated as mentioned above.

2.9.1. Hematology

Beckman Coulter a fully automated cell counter was used to analyze hematological parameters of collected blood samples on 90th and 180th day. Hematological examination includes hemoglobin concentration (HGB), red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Hematocrit (HCT), differential leukocyte count (DLC).

2.9.2. Serum biochemistry

The Blood collected in plain vacutainer was allowed to clot at room temperature. The clotted blood was centrifuged to separate serum. Fully automated biochemical analyzer was used to examine serum biochemical parameters on 90th and 180th day. The biochemistry parameter includes assays for alanine aminotransferase (ALT/SGPT) [15], aspartate amino-transferase (AST/SGOT) [16], alkaline phosphatase (ALP) [17], total bilirubin [18], total protein [19], albumin, globulin [20], creatinine [21], urea [22], BUN (Blood urea nitrogen) [23], blood glucose [24], cholesterol [25], triglyceride [26], albumin/globulin ratio (A/G), potassium, sodium, chloride concentration.

2.9.3. Histopathology

All the surviving animals belonging to different groups except the satellite group were euthanized using carbon dioxide asphyxiation after completion of 180 days of dose treatment. Organs were collected, weighed and processed for histopathological examination. Different organs/tissues collected includes adrenals, aorta, ecem, epididymis, esophagus, eyes, intestine, mesenteric lymph node, mandibular lymph node, muscle, pancreas, pituitary, prostate, seminal vesicles, salivary gland, skin, spinal cord, spleen, sternum, stomach, thyroid and parathyroid gland, testes thymes, trachea, urinary bladder, brain, heart, kidney, liver, lungs, uterine ovary. In satellite group animals were sacrificed after 4 weeks on completion of dose treatment and same procedure was followed as per other group. The tissue sections from animals in control and high dose group were examined microscopically. If any toxicological indication was observed in tissues from high dose group treated animals, then tissues from low dose and mid dose groups were also microscopically examined. All gross lesions and above mentioned tissues were sectioned followed by H and E staining method. The slides were observed under the microscope to note histopathological lesions, if any. As per severity observed, lesions were classified as NAD = No abnormality detected, 1 = Minimal (<1%), 2 = Mild (1–25%), 3 = Moderate (26–50%), 4 = Moderately severe/marked (51–75%), 5 = Severe (76–100%). Lesion distribution was recorded and categorized as focal, multifocal and diffused.

Table 3c

| Parameters               | Group I (Normal Control) | Group II (SKC TD) | Group III (SKC 2.5 TD) | Group IV (SKC 5 TD) | Group V (SKC 5 TD) (Satellite group) | Group VI (Marketed formulation) |
|-------------------------|--------------------------|-------------------|------------------------|---------------------|--------------------------------------|----------------------------------|
| WBC (cells/cmm)         | 10.11 ± 0.70             | 10.31 ± 1.24      | 10.36 ± 1.49           | 10.71 ± 1.37        | 10.20 ± 1.73                        | 10.77 ± 1.11                     |
| RBC (million/mm³)       | 7.82 ± 0.67              | 7.85 ± 0.76       | 8.01 ± 0.79            | 7.78 ± 0.55         | 7.86 ± 0.46                         | 8.27 ± 0.68*                     |
| Hgb (g/dL)              | 14.88 ± 0.61             | 14.58 ± 0.75      | 14.52 ± 0.87           | 14.18 ± 0.57        | 13.96 ± 0.83                        | 14.66 ± 1.17                     |
| MCH (pg)                | 44.75 ± 2.25             | 42.71 ± 2.52      | 45.95 ± 1.03           | 44.96 ± 1.54        | 44.88 ± 3.94                        | 44.66 ± 3.51                     |
| MCV (FL)                | 57.67 ± 6.40             | 54.98 ± 6.85      | 57.81 ± 4.40           | 57.97 ± 3.77        | 57.37 ± 7.60                        | 54.11 ± 3.80                     |
| MCH (pg)                | 19.12 ± 1.46             | 18.73 ± 1.91      | 18.24 ± 1.57           | 18.25 ± 1.10        | 17.78 ± 1.18                        | 17.76 ± 1.21                     |
| MCHC (g/dl)             | 33.32 ± 2.08             | 34.25 ± 2.67      | 31.60 ± 1.73           | 31.50 ± 1.05        | 31.36 ± 3.95                        | 32.85 ± 1.28                     |
| PLT (% × 10³/µL)        | 908.1 ± 134.13           | 919.2 ± 97.06     | 773.6 ± 75.91          | 924.9 ± 94.02       | 921.2 ± 51.94                       | 902.4 ± 34.33                    |
| Neutrophil (%)          | 16.70 ± 2.16             | 17.20 ± 3.65      | 16.90 ± 3.03           | 17.00 ± 1.83        | 17.00 ± 2.45                        | 18.20 ± 2.17                     |
| Lymphocyte (%)          | 79.40 ± 1.90             | 79.30 ± 2.95      | 79.60 ± 2.91           | 79.10 ± 2.13        | 79.60 ± 3.05                        | 77.80 ± 1.79                     |
| Monocyte (%)            | 2.50 ± 1.08              | 2.00 ± 0.82       | 2.20 ± 1.14            | 2.40 ± 1.26         | 2.20 ± 1.10                         | 2.20 ± 0.84                      |
| Eosinophil (%)          | 1.40 ± 0.84              | 1.40 ± 0.84       | 1.30 ± 0.67            | 1.50 ± 0.53         | 1.20 ± 0.84                         | 1.80 ± 0.84                      |
| Basophil (%)            | 0.00 ± 0.00              | 0.00 ± 0.00       | 0.00 ± 0.00            | 0.00 ± 0.00         | 0.00 ± 0.00                         | 0.00 ± 0.00                      |
Table 4a
Effect of oral administration of Shwaskas Chintamani Rasa and Kas Shwas Hari Rasa tablet on serum biochemistry parameters in male rats after 90 days. Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett’s multiple test for comparison. Level of significance P < 0.05.

| Parameters                        | Group I (Normal Control) | Group II (SKC TD) | Group III (SKC 2.5 TD) | Group IV (SKC 5 TD) | Group V (SKC 5 TD) (Satellite group) | Group VI (Marketed formulation) |
|-----------------------------------|--------------------------|-------------------|------------------------|---------------------|--------------------------------------|---------------------------------|
| Albumin (g/dL)                    | 3.456 ± 0.16             | 3.512 ± 0.18      | 3.716 ± 0.21          | 3.639 ± 0.18        | 3.790 ± 0.28                        | 3.748 ± 0.23                   |
| ALP (U/L)                         | 229.1 ± 41.94            | 250.4 ± 44.74     | 213.0 ± 47.08         | 231.4 ± 35.55       | 246.8 ± 40.98                       | 255.6 ± 31.18                  |
| ALT (U/L)                         | 46.19 ± 5.13             | 54.53 ± 8.13      | 54.19 ± 8.94          | 50.56 ± 5.46        | 52.92 ± 5.83                        | 48.18 ± 4.22                   |
| AST (U/L)                         | 147.2 ± 8.77             | 164.8 ± 23.95     | 156.7 ± 25.15         | 160.4 ± 37.70       | 136.8 ± 8.02                        | 142.2 ± 9.40                   |
| T. Bil (mg/dL)                    | 0.1570 ± 0.02            | 0.1580 ± 0.02     | 0.1500 ± 0.02         | 0.1520 ± 0.03       | 0.1440 ± 0.02                       | 0.1620 ± 0.03                  |
| Calcium (mg/dL)                   | 11.99 ± 0.80             | 12.26 ± 1.17      | 11.98 ± 0.37          | 12.18 ± 0.85        | 12.21 ± 0.78                        | 12.08 ± 0.45                   |
| Cholesterol (mg/dL)               | 38.94 ± 4.44             | 38.95 ± 4.92      | 40.04 ± 5.29          | 42.71 ± 4.90        | 43.86 ± 4.78                        | 43.04 ± 3.96                   |
| Creatinine (mg/dL)                | 0.313 ± 0.04             | 0.342 ± 0.03      | 0.363 ± 0.02          | 0.340 ± 0.02        | 0.378 ± 0.06                        | 0.378 ± 0.04                   |
| Glucose (mg/dL)                   | 76.95 ± 8.06             | 78.31 ± 6.04      | 85.17 ± 5.61          | 80.58 ± 4.21        | 76.30 ± 5.60                        | 75.86 ± 5.84                   |
| A/G Ratio                         | 1.51 ± 0.67              | 1.29 ± 0.35       | 1.82 ± 0.64           | 2.12 ± 0.78         | 1.69 ± 0.64                         | 1.34 ± 0.68                    |
| BUN (mg/dL)                       | 17.77 ± 1.51             | 17.29 ± 1.47      | 19.21 ± 2.01          | 19.99 ± 2.51        | 20.88 ± 2.07                        | 18.84 ± 2.10                   |
| Triglycerides (mg/dL)             | 80.97 ± 12.55            | 66.85 ± 17.30     | 70.95 ± 12.17         | 64.17 ± 19.33       | 74.82 ± 9.52                        | 77.58 ± 9.48                   |
| Urea (mg/dL)                      | 38.01 ± 3.25             | 36.99 ± 3.15      | 41.08 ± 4.31          | 42.78 ± 5.38        | 44.68 ± 4.43                        | 40.32 ± 4.49                   |
| Globulin (g/dL)                   | 3.648 ± 0.40             | 3.292 ± 0.35      | 3.857 ± 0.38          | 3.870 ± 0.38        | 3.810 ± 0.42                        | 3.926 ± 0.42                   |
| A/G Ratio                         | 0.875 ± 0.01             | 1.083 ± 0.18      | 0.9720 ± 0.11         | 0.9450 ± 0.10       | 1.002 ± 0.08                        | 0.9620 ± 0.10                  |
| T. Protein (g/dL)                 | 6.20 ± 0.07              | 7.236 ± 0.75      | 6.769 ± 0.61          | 7.143 ± 0.73        | 6.996 ± 0.63                        | 7.220 ± 0.40                   |
| Potassium (mmol/l)                | 165.3 ± 6.20             | 169.8 ± 3.61      | 165.4 ± 3.96          | 167.0 ± 3.09        | 166.7 ± 4.37                        | 165.3 ± 4.14                   |
| Sodium (mmol/l)                   | 103.4 ± 1.86             | 102.9 ± 1.28      | 104.1 ± 2.62          | 102.5 ± 2.19        | 104.4 ± 2.45                        | 103.8 ± 2.18                   |

2.10. Statistical analysis

The data was analyzed using Graph pad prism software. The results were analyzed with one way ANOVA followed by Dunnett’s Multiple Comparison test. All results were expressed as Mean ± SD.

3. Results

3.1. Physiological observations

In both male and female animals from all treated groups, there were no changes observed in general appearance, body position and posture, autonomic nervous system function, motor coordination, reaction during physical handling and environmental stimulation. Also, no signs of tremor, convulsion, abnormal behavior including abnormal vocalization, aggression, lacrimation, salivation and gait pattern were observed in all treated group compared with normal control group.

3.2. Mortality and physiological signs

No treatment related mortality and physiological signs were observed in treated animals.

3.3. Body weight

Body weight of animals was recorded weekly. There were no significant changes observed in body weight of male and female animals in all treatment groups when compared with normal control group (Table 2).

3.4. Biochemical analysis

3.4.1. Hematology

No significant difference in most of the hematological parameters was observed after 90 and 180 days of treatment in all treated group as compared with normal control group. But few
significant variations were observed in some hematological parameters. After 90 days of treatment, significant decrease in monocyte level was observed in male rats of Group III and IV (SKC). After 180 days KSH treatment, increase in RBC level was observed in males compared with normal control group. Both males and females treated with low and high dose of SKC showed significant difference after 180 days in PLT count as compared with normal control. Female rats treated with KSH showed increase in HCT count and decrease in MCH count after 180 days. All the parameters do not carry any toxicological significance (Tables 3a–d).

3.4.2. Serum biochemistry

Serum biochemistry examination did not show any treatment related significant alteration in most of the biochemical parameters after 90 days and 180 days. While some biochemical parameters show significant alterations which does not carry any toxicological significance compared with normal control group (Tables 4a–d).

After 90 days, Albumin level was increased in male rats treated with SKC (mid and satellite) and KSH. The level was also high in female rats treated with SKC (Mid and low).

Significant difference was observed in ALT level in male rats treated with low and mid dose of SKC after 90 days. Creatinine level was increased in male rats belonging to SKC (mid and satellite) as well as KSH treatment group and in female rats belonging to SKC (mid and high) treatment group. Glucose level was significantly increased in male rats treated with SKC mid dose only.

Male rats from SKC mid dose group and female rats from SKC mid and high dose group showed increase in total protein level. Level of Blood urea nitrogen was increased in male satellite group (SKC 2.5 TD) on day 180.

Table 4d

Effect of oral administration of Shwaskas Chintamani Rasa and Kas Shwas Hari Rasa tablet on serum biochemistry parameters in female rats after 180 days. Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett’s multiple test for comparison. Level of significance #P < 0.01.

| Parameters   | Group I (Normal formulation) | Group II (SKC TD) | Group III (SKC 2.5 TD) | Group IV (SKC 5 TD) | Group V (SKC 5 TD Satellite group) | Group VI (Marketed formulation) |
|--------------|-------------------------------|-------------------|------------------------|---------------------|----------------------------------|---------------------------------|
| Albumin (g/dL) | 3.80 ± 0.32                   | 3.68 ± 0.20       | 3.87 ± 0.31            | 3.92 ± 0.25         | 3.67 ± 0.30                      | 4.01 ± 0.24                     |
| ALP (U/L)    | 261 ± 53.47                   | 274.2 ± 27.8      | 260.1 ± 39.5           | 289.8 ± 40.6        | 214.6 ± 40.3                     | 270.8 ± 57.7                    |
| ALT (U/L)    | 49.65 ± 7.29                  | 46.15 ± 5.30      | 43.98 ± 4.34           | 49.03 ± 4.01        | 46.82 ± 5.84                     | 49.00 ± 3.08                    |
| AST (U/L)    | 151 ± 21 ± 11.06              | 154 ± 20 ± 10.6   | 143 ± 48 ± 8.25        | 156 ± 67 ± 2.72     | 146 ± 46 ± 4.94                   | 147 ± 46 ± 13.0                 |
| T. Bil (mg/dL)| 0.16 ± 0.04                   | 0.13 ± 0.04       | 0.15 ± 0.03            | 0.17 ± 0.03         | 0.15 ± 0.04                      | 0.14 ± 0.05                     |
| Calcium (mg/dL)| 11.77 ± 0.50                 | 10.92 ± 0.78      | 11.43 ± 0.67           | 11.35 ± 0.71        | 11.37 ± 1.22                     | 10.90 ± 0.54                    |
| Cholesterol (mg/dL)| 41.70 ± 29.11 | 42.77 ± 5.40 | 43.03 ± 5.32           | 46.08 ± 4.33        | 45.38 ± 7.18                     | 43.80 ± 5.36                    |
| Creatinine (mg/dL) | 0.32 ± 0.04      | 0.33 ± 0.05      | 0.35 ± 0.04            | 0.36 ± 0.04         | 0.34 ± 0.05                      | 0.31 ± 0.05                     |
| Glucose (mg/dL) | 77.47 ± 9.30                  | 75.35 ± 7.78      | 75.84 ± 8.36           | 74.35 ± 6.53        | 79.74 ± 9.64                     | 78.94 ± 6.88                    |
| T. Protein (g/dL)| 7.26 ± 0.63                   | 7.49 ± 0.57       | 7.54 ± 0.44            | 7.34 ± 0.51         | 7.71 ± 0.49                      | 7.45 ± 0.57                     |
| Triglycerides (mg/dL) | 83.47 ± 11.88              | 86.13 ± 11.36     | 83.26 ± 13.63          | 87.22 ± 11.32       | 91.02 ± 12.09                     | 87.94 ± 10.24                    |
| Urea (mg/dL) | 39.31 ± 3.58                  | 35.40 ± 3.54      | 37.53 ± 2.45           | 37.57 ± 2.86        | 36.20 ± 5.23                     | 36.26 ± 5.98                    |
| Globulin (g/dL) | 3.36 ± 0.59                   | 3.80 ± 0.55       | 3.66 ± 0.50            | 3.42 ± 0.46         | 4.04 ± 0.41                      | 3.44 ± 0.80                     |
| A/G Ratio   | 1.22 ± 0.24                   | 0.99 ± 0.19       | 1.08 ± 0.21            | 1.17 ± 0.21         | 0.92 ± 0.13                      | 1.23 ± 0.36                     |
| BUN (mg/dL) | 18.22 ± 1.72                  | 16.54 ± 1.07      | 17.54 ± 1.15           | 17.56 ± 1.34        | 16.92 ± 2.44                     | 16.94 ± 2.79                    |
| Albumin (g/dL) | 166 ± 0.31                    | 163.79 ± 3.51     | 164.30 ± 3.17          | 164.65 ± 2.98       | 163.94 ± 4.10                     | 165.70 ± 3.53                    |
| Cholesterol (mg/dL) | 103.86 ± 2.09                 | 104.06 ± 2.33     | 103.69 ± 2.30          | 105.14 ± 1.20       | 103.44 ± 1.14                     | 103.42 ± 1.20                    |

C. S. Waghmare, S. Bidve, R. V. Gudi et al. Journal of Ayurveda and Integrative Medicine 13 (2022) 100615
3.4.3. Histopathology

The histopathological examination was carried out on both male and female animals of high dose group and normal control group. Both male and female animals did not show any lesions of pathological significance in treated high dose group when compared with normal control group. Therefore, the examination was not carried out on low and mid dose groups (Ref Figs. 1–4).

4. Discussion

Most of the ayurvedic formulations contains heavy metals like mercury, sulfur, mica and iron. Toxicity caused by these metals is a major concern. Various Shodhan (Purification) processes were used for the purification of toxic metals to achieve their therapeutic values [27]. Different Bhasmas used in ayurvedic formulations are safe if prepared as per ayurvedic granth (text...
reference) and prescribed with several Anupanas (vehicle) [28,29]. SKC was found to be considerably safe in a neurological study [12]. In the present study, Shwaskas Chintamani Rasa (SKC) and Kas Shwas Hari Rasa (KSH) tablets were evaluated for repeated dose chronic toxicity for 180 days.

The important factor to evaluate in health of an animal is changes in body weight. Decrease in body weight is usually the main sign showing the beginning of an adverse effect. The dose is considered to be toxic when it causes more than 10% loss of body weight in animals [30].

On administration of SKC and KSH, there was no significant difference observed in all treated groups when compared with normal control group. This indicates the absence of toxic effect of SKC and KSH on chronic administration. There was no alteration detected in physiological signs in all treated groups compared with normal control group. As well as no treatment related mortality observed in animals.

Treatment of KSH and SKC shows decrease in Monocyte and increase in RBC levels after 90 days. But these changes are within physiological limits and do not carry any toxicological significance.
After completion of experiment, level of RBC and Monocyte was within normal range in all groups.

Level of PLT, HCT and MCH was also changed after 180 days in some treated group as compared to normal control group. But all the variations are in physiological limits and do not carry any toxicological effects. These biochemical changes observed may be due to presence of metals in KSH and SKC formulations. Several studies have shown elevated serum biochemical parameters due to exposure of mercury in ayurvedic formulations which are within physiological limits and do not carry any toxicological effects [31,32].

After 90 days of drug administration the increase inAlbumin, Creatinine, Glucose, Total protein, ALT, and BUN level was seen in some groups. Level of AST was decreased after 180 days of drug administration (female high SKC dose group). The changes are within normal range and do not represent any toxicological effect.

At termination of study both males and females of normal control and high dose group were sacrificed for histopathological examination of various organs. All the animal belonging to drug administered group did not show any lesions of pathological significance.
KSH and SKC used in this study also did not show any toxicological effect.

5. Conclusion

The male and female Wistar rats well tolerated the chronic administration of Shwaskas Chintamani Rasa tablets and Kas Shwas Hari Rasa tablets for consecutive period of 180 days. There was no treatment related adverse alterations observed in physiological signs, body weight, hematology, serum biochemistry and histopathology analysis. Based on these findings, the NOAEL (No observed adverse effect level) for Shwaskas Chintamani Rasa (5 times of therapeutic dose) and Kas Shwas Hari Rasa (therapeutic dose) tablets in wistar rats was found to be safe.

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Credit author statement

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Shivcharan Bidve: Supervision, Data Curation, Resources.
R. V. Gudi: Conceptualization, Supervision, Resources, Project administration.
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Megha L.NALAWADE: Methodology, Writing - Review & Editing, Supervision.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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C.S. Waghmare, S. Bidve, R.V. Gudi et al. Journal of Ayurveda and Integrative Medicine 13 (2022) 100615

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