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Original Research Article

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.

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 sub-continental 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 (calcined gold), Shodhit Parad (purified mercury), Shodhit Gandhak (purified sulfur), Suvarnamakshik Bhasma (Chalcopyrite), Abhraka Bhasma (calcined mica), Loha Bhasma (calcined iron), and Mouktik Bhasma (calcined pearl). All these ingredients were processed in Kantakari Swarasa (*Solanum surattense*), Godugdha (goat milk), Yashtimadhu Kwath (*Glycrrhiza 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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Table 1
Group-wise distribution of experimental Wistar rats.

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 (5TD) SKC	290	20	20
V	Satellite group (5TD) 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.

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), Sootashekhar Rasa and Taleesadi Choorna. All these ingredients were processed in Vasa Kwath (*Adhatoda vasica Linn.*). These tables 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.

2.4. Preparation of dose

The powder of Shwaskas Chintamani Rasa and Kas Shwas Hari Rasa tablets was prepared using mortar and pestle. The powder is

Table 2
Effect of oral administration of Shwaskas Chintamani Rasa and Kas Shwas Hari Rasa tablet on body weight in male (A) and female (B) animals.

(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	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
(B) Body weight (Female)		
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

Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison.

Table 3a
Effect of oral administration of Shwaskas Chintamani Rasa and Kas-shwas Hari Rasa tablet on hematology parameters in male 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)
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/mm^3)	7.975 ± 0.56	7.67 ± 0.67	7.557 ± 0.65	8.046 ± 0.28	7.836 ± 0.63	7.994 ± 0.20
Hgb (g/dL)	14.83 ± 0.89	14.51 ± 0.96	14.41 ± 0.72	14.68 ± 0.61	14.52 ± 0.90	14.78 ± 0.76
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)	18.66 ± 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 ± 2.08	32.81 ± 1.65	33.51 ± 2.40	31.66 ± 0.43	32.36 ± 1.61	32.74 ± 2.88
PLT (×10^3/μL)	821.4 ± 90.7	882.6 ± 74.2	912.2 ± 66.7	863.3 ± 84.9	901.6 ± 77.1	908.8 ± 71.2
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†	1.600 ± 1.14	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

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., predosing, during dosing and post-dosing for the following physiological observations:

General appearance, body position and posture, autonomic nervous system function, motor coordination, reaction during physical handling, environmental stimulation, tremor, convulsion, 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 hematology analysis EDTA was used as an anticoagulant. For serum biochemical analysis blood was collected in plain vacutainer.

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.

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)	11.01 ± 1.45	10.65 ± 1.27	10.19 ± 1.13	10.34 ± 0.95	10.28 ± 0.87	10.42 ± 0.61
RBC (million/mm^3)	7.258 ± 0.38	7.327 ± 0.48	7.264 ± 0.51	7.153 ± 0.18	7.330 ± 0.35	7.472 ± 0.20
Hgb (g/dL)	14.16 ± 1.01	14.90 ± 0.86	14.04 ± 0.88	14.17 ± 0.53	14.16 ± 0.45	14.42 ± 0.40
HCT (%)	42.53 ± 3.13	42.15 ± 2.55	40.46 ± 2.26	42.63 ± 3.06	43.12 ± 1.78	45.84 ± 1.41
MCV (fL)	58.54 ± 1.97	57.70 ± 4.41	55.98 ± 5.30	59.61 ± 4.40	58.86 ± 2.86	61.34 ± 1.29
MCH (pg)	19.50 ± 0.89	20.36 ± 1.18	19.46 ± 0.73	19.81 ± 0.59	19.38 ± 1.25	19.30 ± 0.44
MCHC (g/dL)	33.33 ± 1.25	35.47 ± 3.20	35.00 ± 3.16	33.36 ± 2.09	32.92 ± 1.79	31.48 ± 0.31
PLT (×10^3/μL)	887.9 ± 109.8	859.7 ± 84.9	889.9 ± 69.1	925.7 ± 91.0	817.8 ± 93.7	931.0 ± 72.5
Neutrophil (%)	17.60 ± 3.40	17.20 ± 3.64	18.40 ± 2.50	18.80 ± 3.52	19.40 ± 3.20	18.40 ± 2.07
Lymphocyte (%)	79.70 ± 3.36	79.00 ± 3.19	77.50 ± 2.83	78.30 ± 3.05	77.60 ± 4.09	78.20 ± 2.28
Monocyte (%)	1.300 ± 0.67	1.900 ± 0.87	2.700 ± 0.94	1.700 ± 1.16	1.600 ± 0.54	2.000 ± 1.0
Eosinophil (%)	1.400 ± 0.69	1.900 ± 0.87	1.400 ± 1.17	1.200 ± 1.03	1.400 ± 1.14	1.400 ± 0.54
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 3c
Effect of oral administration of Shwaskas Chintamani Rasa and Kas-shwas Hari Rasa tablet on hematology parameters in male 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 < 0.05; *P < 0.0001.

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/mm^3)	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	14.16 ± 0.57	13.96 ± 0.83	14.66 ± 1.17
HCT (%)	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 ± 4.90	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 (×10^3/μL)	908.1 ± 134.13	919.2 ± 97.06	773.6 ± 75.9†	924.9 ± 94.02	923.2 ± 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

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 amino-transferase (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), calcium [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, cecum, 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 thymus, trachea, urinary bladder, brain, heart, kidney, liver, lungs, uterus 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 3d
Effect of oral administration of Shwaskas Chintamani Rasa and Kas Shwas Hari Rasa tablet on hematology parameters in female 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 < 0.05; #P < 0.01; *P < 0.0001.

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)	10.40 ± 1.06	10.45 ± 1.07	10.59 ± 1.59	10.90 ± 1.07	10.10 ± 0.37	10.38 ± 0.79
RBC (million/mm^3)	7.79 ± 0.63	7.89 ± 0.82	7.43 ± 0.45	7.54 ± 0.61	7.56 ± 0.78	8.30 ± 0.53
Hgb (g/dL)	14.79 ± 0.78	14.33 ± 0.59	14.04 ± 0.83	13.98 ± 0.49	13.96 ± 0.97	13.84 ± 0.59
HCT (%)	44.87 ± 1.57	40.80 ± 1.75*	42.33 ± 2.18†	43.65 ± 1.46	44.86 ± 2.81	42.43 ± 1.42
MCV (fL)	57.98 ± 5.30	52.30 ± 6.74	57.07 ± 3.62	58.20 ± 4.30	59.91 ± 7.57	51.29 ± 3.75
MCH (pg)	19.09 ± 1.64	18.36 ± 2.36	18.96 ± 1.73	18.65 ± 1.51	18.62 ± 2.14	16.72 ± 1.14*
MCHC (g/dL)	33.02 ± 2.43	35.19 ± 2.14	33.27 ± 2.88	32.03 ± 0.79	31.16 ± 2.11	32.62 ± 0.86
PLT (×10^3/μL)	942.5 ± 89.58	930.1 ± 60.50	838.6 ± 86.1†	916.0 ± 72.27	920.6 ± 48.85	909.0 ± 85.40
Neutrophil (%)	17.60 ± 3.20	17.80 ± 3.05	19.00 ± 3.40	17.20 ± 2.62	19.20 ± 2.17	16.60 ± 1.14
Lymphocyte (%)	78.90 ± 3.21	78.80 ± 3.19	77.50 ± 2.84	79.00 ± 2.31	77.20 ± 1.92	79.60 ± 1.95
Monocyte (%)	2.10 ± 0.99	1.90 ± 0.88	2.00 ± 1.15#	2.20 ± 1.03	2.40 ± 1.14	2.20 ± 0.84
Eosinophil (%)	1.40 ± 0.97	1.50 ± 1.50	1.50 ± 1.08	1.60 ± 0.97	1.20 ± 0.84	1.60 ± 1.14
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	231.5 ± 35.55	246.8 ± 40.98	255.6 ± 31.18
ALT (U/L)	46.19 ± 5.13	54.53 ± 8.15†	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	12.31 ± 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
T. Protein (g/dL)	7.104 ± 0.39	6.804 ± 0.30	7.573 ± 0.42†	7.517 ± 0.26	7.402 ± 0.42	7.474 ± 0.42
Triglycerides (mg/dL)	80.97 ± 12.55	66.85 ± 17.30	70.95 ± 12.17	64.96 ± 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	3.878 ± 0.30	3.810 ± 0.42	3.926 ± 0.42
A/G Ratio	0.9590 ± 0.12	1.083 ± 0.18	0.9720 ± 0.11	0.9450 ± 0.10	1.002 ± 0.08	0.9620 ± 0.10
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
Potassium (mmol/l)	6.653 ± 0.67	7.236 ± 0.75	6.769 ± 0.61	7.143 ± 0.73	6.996 ± 0.63	7.220 ± 0.40
Sodium (mmol/l)	165.5 ± 6.20	169.8 ± 3.61	165.4 ± 3.96	167.0 ± 3.09	166.7 ± 4.37	165.5 ± 4.14
Chloride (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

Table 4b
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 < 0.05; #P < 0.01.

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)	3.620 ± 0.27	3.933 ± 0.23†	4.057 ± 0.27#	3.919 ± 0.29	3.674 ± 0.24	3.878 ± 0.11
ALP (U/L)	236.1 ± 32.41	195.8 ± 22.8†	219.2 ± 37.52	244.3 ± 35.17	241.7 ± 45.22	232.5 ± 33.20
ALT (U/L)	45.11 ± 5.04	50.18 ± 6.31	51.06 ± 8.00	47.67 ± 7.96	49.46 ± 2.44	48.22 ± 6.78
AST (U/L)	145.8 ± 8.55	159.7 ± 12.73	150.7 ± 17.46	150.0 ± 37.03	138.8 ± 5.37	140.3 ± 8.15
T. Bil (mg/dL)	0.1560 ± 0.02	0.1770 ± 0.04	0.1540 ± 0.03	0.1890 ± 0.02	0.1560 ± 0.02	0.1660 ± 0.03
Calcium (mg/dL)	11.90 ± 1.05	12.03 ± 0.72	12.40 ± 0.66	12.19 ± 0.59	12.49 ± 0.68	11.84 ± 0.46
Cholesterol (mg/dL)	40.17 ± 4.72	44.93 ± 7.83	43.29 ± 5.79	46.55 ± 5.66	45.96 ± 5.35	44.68 ± 5.49
Creatinine (mg/dL)	0.3270 ± 0.03	0.3610 ± 0.04	0.4060 ± 0.03†	0.380 ± 0.04†	0.3740 ± 0.04	0.3740 ± 0.04
Glucose (mg/dL)	75.90 ± 8.07	70.52 ± 5.91	75.51 ± 8.71	77.76 ± 5.78	80.46 ± 7.31	73.60 ± 4.84
T. Protein (g/dL)	7.277 ± 0.65	7.482 ± 0.49	8.009 ± 0.32#	7.945 ± 0.47†	7.594 ± 0.26	7.742 ± 0.40
Triglycerides (mg/dL)	82.86 ± 14.25	81.84 ± 23.28	79.77 ± 14.77	83.42 ± 35.06	79.98 ± 15.08	85.58 ± 13.09
Urea (mg/dL)	38.97 ± 4.52	39.87 ± 4.23	40.77 ± 3.80	39.43 ± 6.61	42.82 ± 3.56	45.56 ± 6.69
Globulin (g/dL)	3.657 ± 0.56	3.549 ± 0.59	3.952 ± 0.39	4.026 ± 0.64	3.920 ± 0.31	4.004 ± 0.57
A/G Ratio	1.013 ± 0.17	1.157 ± 0.32	1.041 ± 0.16	1.004 ± 0.21	0.9440 ± 0.12	0.9840 ± 0.13
BUN (mg/dL)	17.96 ± 2.27	18.64 ± 1.97	19.09 ± 1.74	18.42 ± 3.08	20.01 ± 1.66	21.30 ± 3.13
Potassium (mmol/l)	6.956 ± 0.39	6.713 ± 0.43	6.846 ± 0.69	7.051 ± 0.61	7.222 ± 0.32	7.250 ± 0.44
Sodium (mmol/l)	163.0 ± 3.96	165.0 ± 6.78	166.2 ± 4.44	164.2 ± 4.31	168.2 ± 5.42	165.9 ± 3.92
Chloride (mmol/l)	103.0 ± 1.96	103.9 ± 2.04	104.1 ± 2.22	102.9 ± 1.63	103.7 ± 2.20	103.6 ± 2.48

Table 4c
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.

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)	3.80 ± 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)	266.1 ± 53.47	274.2 ± 27.8	260.1 ± 39.5	289.8 ± 40.6	261.4 ± 40.58	270.8 ± 57.76
ALT (U/L)	49.65 ± 7.29	46.15 ± 5.10	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	156.08 ± 6.72	144.46 ± 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 ± 2.91	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	0.33 ± 0.05
Glucose (mg/dL)	77.47 ± 9.30	75.35 ± 7.78	78.54 ± 8.36	74.35 ± 6.53	79.74 ± 9.64	78.94 ± 6.86
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)	39.41 ± 3.58	35.40 ± 35.40	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
Potassium (mmol/l)	6.76 ± 0.47	6.68 ± 0.65	6.86 ± 0.36	7.02 ± 0.56	6.61 ± 0.48	7.09 ± 0.56
Sodium (mmol/l)	166.10 ± 3.61	163.79 ± 3.51	164.30 ± 3.17	164.65 ± 2.98	163.94 ± 4.10	165.70 ± 3.53
Chloride (mmol/l)	103.86 ± 2.09	104.06 ± 2.33	103.69 ± 2.30	105.14 ± 1.20	103.44 ± 1.44	104.32 ± 1.20

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 variations do not carry any toxicological significance (Tables 3a–d).

3.4.2. Serum biochemistry

Serum biochemical 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 after 3 months. Decrease in AST level was observed in female high dose group after 180 days.

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 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)	3.83 ± 0.27	4.01 ± 0.18	3.82 ± 0.25	3.58 ± 0.30	3.81 ± 0.44	3.85 ± 0.39
ALP (U/L)	245.83 ± 46.39	234.96 ± 36.01	243.48 ± 39.70	237.16 ± 38.61	233.32 ± 80.83	305.50 ± 126.77
ALT (U/L)	48.25 ± 5.46	50.80 ± 6.13	50.97 ± 5.44	46.79 ± 4.70	45.24 ± 7.15	48.16 ± 7.41
AST (U/L)	146.89 ± 15.31	142.06 ± 10.58	150.38 ± 7.37	131.32 ± 6.2#	147.30 ± 11.80	145.22 ± 6.56
T. Bil (mg/dL)	0.18 ± 0.02	0.15 ± 0.04	0.16 ± 0.03	0.17 ± 0.04	0.14 ± 0.03	0.17 ± 0.05
Calcium (mg/dL)	12.03 ± 0.56	11.75 ± 0.62	11.73 ± 0.47	11.93 ± 0.35	11.93 ± 0.80	11.62 ± 0.92
Cholesterol (mg/dL)	44.58 ± 5.66	44.67 ± 5.12	49.91 ± 4.83	46.63 ± 5.41	44.88 ± 6.59	45.96 ± 5.52
Creatinine (mg/dL)	0.34 ± 0.05	0.32 ± 0.03	0.34 ± 0.04	0.32 ± 0.04	0.33 ± 0.06	0.34 ± 0.04
Glucose (mg/dL)	74.41 ± 5.37	77.28 ± 10.71	78.69 ± 9.09	81.42 ± 7.83	80.72 ± 7.81	79.72 ± 10.50
T. Protein (g/dL)	7.33 ± 0.43	7.47 ± 0.31	7.36 ± 0.40	7.42 ± 0.44	7.50 ± 0.43	7.56 ± 0.48
Triglycerides (mg/dL)	85.87 ± 14.26	88.73 ± 11.19	83.61 ± 11.65	88.76 ± 12.38	78.42 ± 11.89	79.26 ± 8.74
Urea (mg/dL)	38.13 ± 3.11	37.65 ± 2.57	36.79 ± 3.09	37.64 ± 3.19	39.56 ± 5.63	35.86 ± 3.12
Globulin (g/dL)	3.34 ± 0.51	3.46 ± 0.28	3.54 ± 0.54	3.84 ± 0.41	3.69 ± 0.22	3.71 ± 0.49
A/G Ratio	1.19 ± 0.22	1.17 ± 0.12	1.11 ± 0.23	0.94 ± 0.14	1.04 ± 0.15	1.05 ± 0.19
BUN (mg/dL)	17.52 ± 1.37	17.59 ± 1.20	17.19 ± 1.44	17.59 ± 1.49	18.49 ± 2.63	16.76 ± 1.46
Potassium (mmol/l)	6.88 ± 0.34	7.04 ± 0.53	6.70 ± 0.56	6.80 ± 0.61	7.10 ± 0.59	6.66 ± 0.41
Sodium (mmol/l)	164.01 ± 2.38	166.11 ± 2.72	165.32 ± 3.05	166.07 ± 2.78	165.56 ± 3.16	166.72 ± 2.51
Chloride (mmol/l)	102.89 ± 2.52	103.85 ± 2.39	104.36 ± 2.42	104.67 ± 1.88	103.98 ± 2.60	103.24 ± 2.71

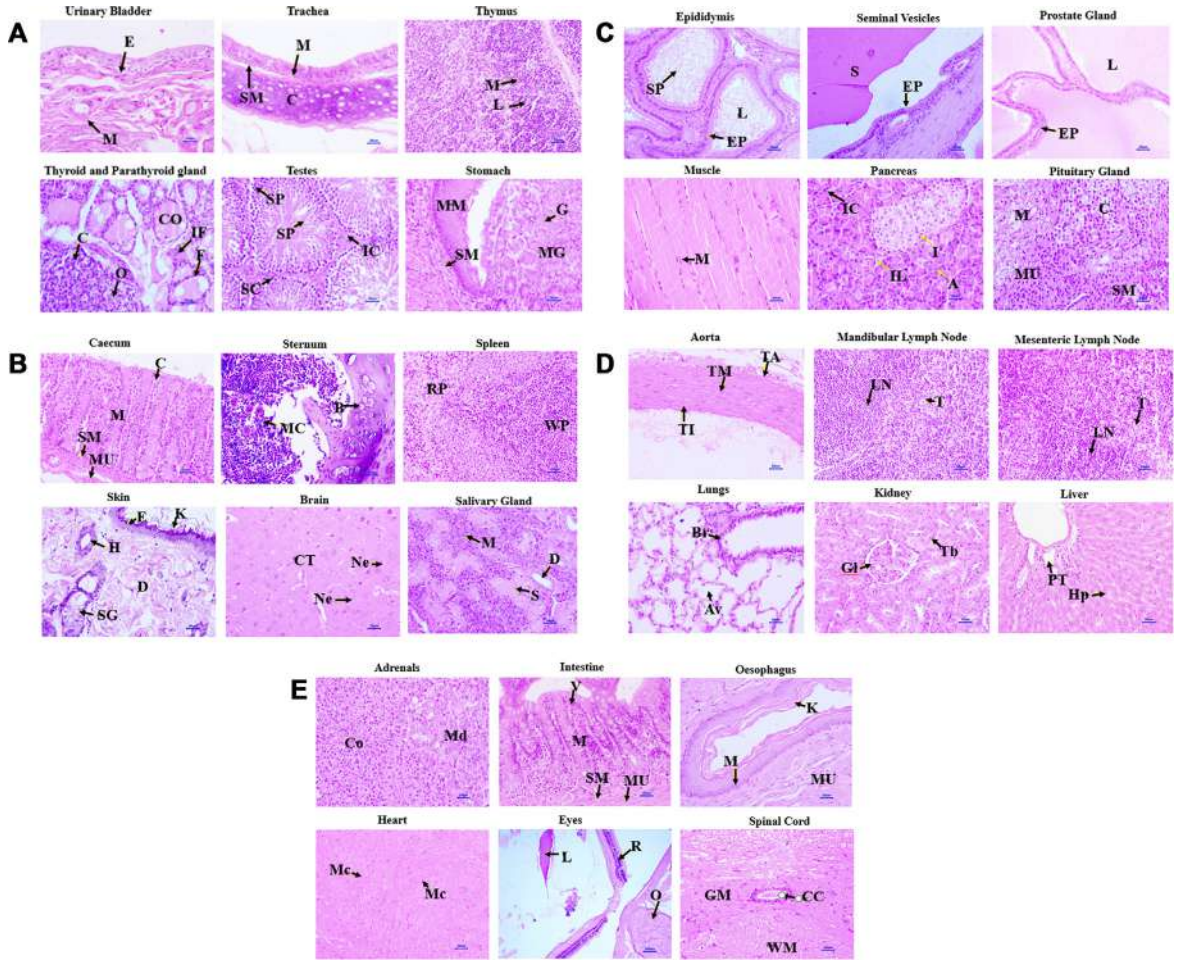

Fig. 1. Histopathology Images of Male Normal Control group. **A**: Normal Control Male (H and E Staining): **Urinary Bladder:** Showing normal histology, epithelium (E) and muscle (M), **Trachea:** Showing normal histology, tracheal cartilage (C), mucosa (M) and submucosa (SM), **Thymus:** Showing normal histology, lymphocytes (L) at medulla (M), **Thyroid and Parathyroid gland:** Showing normal histology, Chief cell (C), Oxyphil cell (O) at parathyroid gland and Follicular lamina filled with acidophilic colloid (CO), Follicular epithelium (F), Interfollicular cells (IF), **Testes:** Showing normal histology of seminiferous tubule, Spermatid (SP), Interstitial cells (IC), Sertoli Cell (SC), **Stomach:** Showing normal histology, mucosa at muscular stomach (MM), mucosa at glandular stomach (MG), submucosa (SM), gland (G). **B**: Normal Control Male (H and E Staining): **Cecum:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU), **Sternum:** Showing normal histology, cells at bone marrow (MC), bone (B), **Spleen:** Showing normal histology, White Pulp (WP), Red Pulp (RP), **Skin:** Showing normal histology, Epithelium (E), sebaceous gland (SG), keratin (K), dermis (D), hair follicle (H), **Brain:** Showing normal histology, neurons (Ne) at cortex (CT), **Salivary gland:** Showing normal histology, mucous gland acini (M), serous gland acini (S), duct (D). **C**: Normal Control Male (H and E Staining): **Epididymis:** Showing normal histology, Sperm (SP), Lumen (L), Epithelium (EP), **Seminal Vesicles:** Showing normal histology, Seminal fluid (S), Epithelium (EP), **Prostate Gland:** Showing normal histology, Lumen with prostatic fluid (L), Epithelium (EP), **Muscle:** Showing normal histology, myocyte (M) at muscle fiber, **Pancreas:** Showing normal histology, acini (A), interlobular duct (IL), intercalated duct (IC), Islets of Langerhans (I), **Pituitary Gland:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU). **D**: Normal Control Male (H and E Staining): **Aorta:** Showing normal histology, Tunic adventitia (TA), Tunica media (TM), Tunica interna (TI), **Mandibular Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Mesenteric Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Lungs:** Showing normal histology, Bronchi (Br) and Alveoli (Av), **Kidney:** Showing normal histology, Glomerulus (Gl), Tubule (Tb), **Liver:** Showing normal histology, Portal triad (PT), Hepatocyte (Hp). **E**: Normal Control Male (H and E Staining): **Adrenals:** Showing normal histology, Cortex (Co) and Medulla (Md), **Intestine:** Showing normal histology, Mucosa (M), Villi (V), Submucosa (SM), Muscularis (MU), **Esophagus:** Showing normal histology, Mucosa (M), keratin layer (K), Muscularis (MU), **Heart:** Showing normal histology, Myocyte (Mc), **Eyes:** Showing normal histology, Lens (L), Retina (R), Optic nerve (O), **Spinal Cord:** Showing normal histology, Central canal (CC), Gray matter (GM), White matter (WM).

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

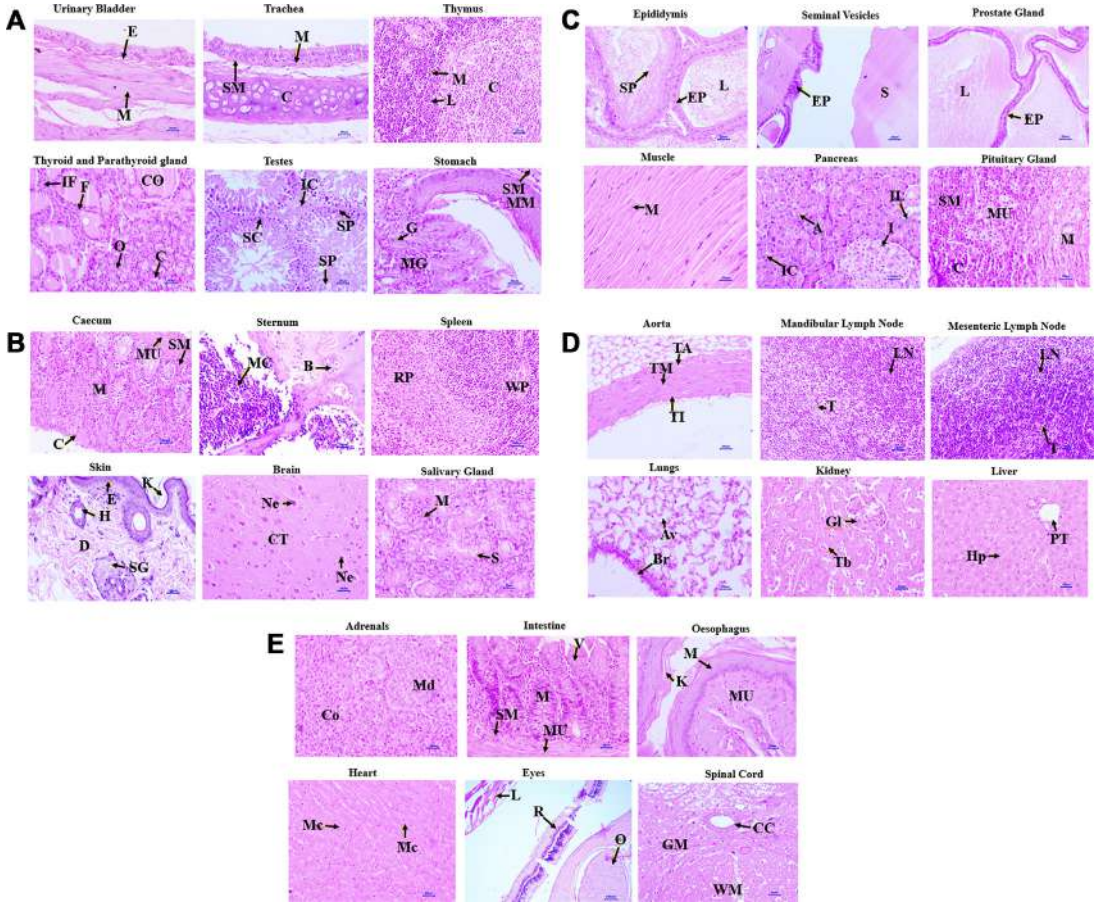

Fig. 2. Histopathology Images of Male High dose group. **A**: High Dose Group Male (H and E staining): Urinary Bladder: Showing normal histology, epithelium (E) and muscle (M), **Trachea:** Showing normal histology, tracheal cartilage (C), mucosa (M) and submucosa (SM), **Thymus:** Showing normal histology, lymphocytes (L) at medulla (M) and cortex (C), **Thyroid and Parathyroid gland:** Showing normal histology, Chief cell (C), Oxyphil cell (O) at parathyroid gland and Follicular lamina filled with acidophilic colloid (CO), Follicular epithelium (F), Interfollicular cells (IF), **Testes:** Showing normal histology of seminiferous tubule, Spermatid (SP), Interstitial cells (IC), Sertoli Cell (SC), **Stomach:** Showing normal histology, mucosa at muscular stomach (MM), mucosa at glandular stomach (MG), submucosa (SM), gland (G). **B**: High Dose Group Male (H and E staining): **Cecum:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU), **Sternum:** Showing normal histology, cells at bone marrow (MC), bone (B), **Spleen:** Showing normal histology, White Pulp (WP), Red Pulp (RP), **Skin:** Showing normal histology, Epithelium (E), sebaceous gland (SG), keratin (K), dermis (D), hair follicle (H), **Brain:** Showing normal histology, neurons (Ne) at cortex (CT), **Salivary gland:** Showing normal histology, mucous gland acini (M), serous gland acini (S). **C**: High Dose Group Male (H and E staining): Epididymis: Showing normal histology, Sperm (SP), Lumen (L), Epithelium (EP), **Seminal Vesicles:** Showing normal histology, Seminal fluid (S), Epithelium (EP), **Prostate Gland:** Showing normal histology, Lumen with prostatic fluid (L), Epithelium (EP), **Muscle:** Showing normal histology, myocyte (M) at muscle fiber, **Pancreas:** Showing normal histology, acini (A), interlobular duct (IL), intercalated duct (IC), Islets of Langerhans (I), **Pituitary Gland:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU). **D**: High Dose Group Male (H and E staining): **Aorta:** Showing normal histology, Tunic adventitia (TA), Tunica media (TM), Tunica interna (TI), **Mandibular Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Mesenteric Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Lungs:** Showing normal histology, Bronchi (Br) and Alveoli (Av), **Kidney:** Showing normal histology, Glomerulus (Gl), Tubule (Tb), **Liver:** Showing normal histology, Portal triad (PT), Hepatocyte (Hp). **E**: High Dose Group Male (H and E staining): **Adrenals:** Showing normal histology, Cortex (Co) and Medulla (Md), **Intestine:** Showing normal histology, Mucosa (M), Villi (V), Submucosa (SM), Muscularis (MU), **Esophagus:** Showing normal histology, Mucosa (M), keratin layer (K), Muscularis (MU), **Heart:** Showing normal histology, Myocyte (Mc), **Eyes:** Showing normal histology, Lens (L), Retina (R), Optic nerve (O), **Spinal Cord:** Showing normal histology, Central canal (CC), Gray matter (GM), White matter (WM).

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.

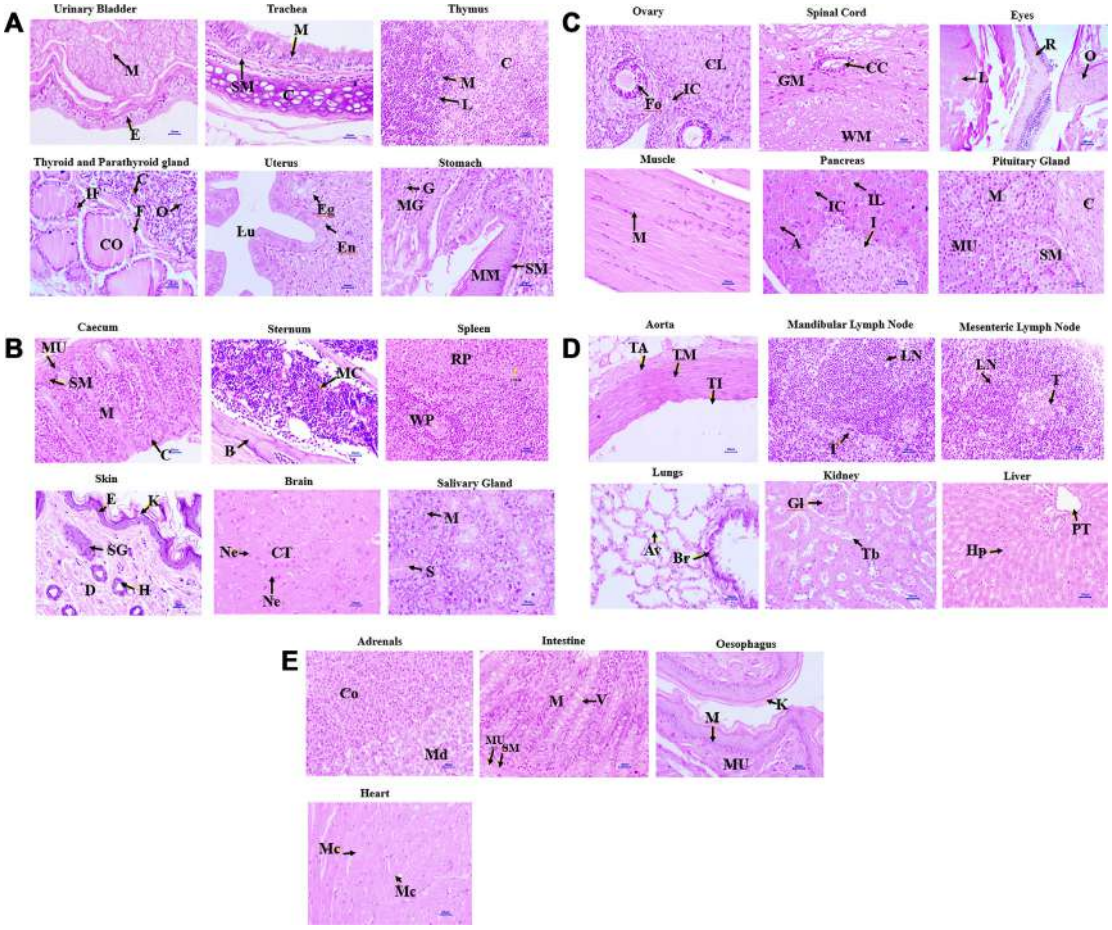

Fig. 3. Histopathology Images of Female Normal Control group. **A**: Normal Control Female (H and E Staining): **Urinary Bladder:** Showing normal histology, epithelium (E) and muscle (M), **Trachea:** Showing normal histology, tracheal cartilage (C), mucosa (M) and submucosa (SM), **Thymus:** Showing normal histology, lymphocytes (L) at medulla (M) and cortex (C), **Thyroid and Parathyroid gland:** Showing normal histology, Chief cell (C), Oxyphil cell (O) at parathyroid gland and Follicular lamina filled with acidophilic colloid (CO), Follicular epithelium (F), Interfollicular cells (IF), **Uterus:** Showing normal histology, endometrium (En), endometrial glands (Eg), Lumen (Lu), **Stomach:** Showing normal histology, mucosa at muscular stomach (MM), mucosa at glandular stomach (MG), submucosa (SM), gland (G). **B**: Normal Control Female (H and E Staining): **Cecum:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU), **Sternum:** Showing normal histology, cells at bone marrow (MC), bone (B), **Spleen:** Showing normal histology, White Pulp (WP), Red Pulp (RP), **Skin:** Showing normal histology, Epithelium (E), sebaceous gland (SG), keratin (K), dermis (D), hair follicle (H), **Brain:** Showing normal histology, neurons (Ne) at cortex (CT), **Salivary gland:** Showing normal histology, mucous gland acini (M), serous gland acini (S). **C**: Normal Control Female (H and E Staining): **Ovary:** Showing normal histology, Follicle (Fo), Interstitial cells (IC) and Corpus luteum (CL), **Spinal Cord:** Showing normal histology, Central canal (CC), Gray matter (GM), White matter (WM), **Eyes:** Showing normal histology, Lens (L), Retina (R), Optic nerve (O), **Muscle:** Showing normal histology, myocyte (M) at muscle fiber, **Pancreas:** Showing normal histology, acini (A), interlobular duct (IL), intercalated duct (IC), Islets of Langerhans (I), **Pituitary Gland:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU). **D**: Normal Control Female (H and E Staining): **Aorta:** Showing normal histology, Tunic adventitia (TA), Tunica media (TM), Tunica interna (TI), **Mandibular Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Mesenteric Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Lungs:** Showing normal histology, Bronchi (Br) and Alveoli (Av), **Kidney:** Showing normal histology, Glomerulus (Gl), Tubule (Tb), **Liver:** Showing normal histology, Portal triad (PT), Hepatocyte (Hp). **E**: Normal Control Female (H and E Staining): **Adrenals:** Showing normal histology, Cortex (Co) and Medulla (Md), **Intestine:** Showing normal histology, Mucosa (M), Villi (V), Submucosa (SM), Muscularis (MU), **Esophagus:** Showing normal histology, Mucosa (M), keratin layer (K), Muscularis (MU), **Heart:** Showing normal histology, Myocyte (Mc).

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 toxicity. 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 in Albumin, 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.

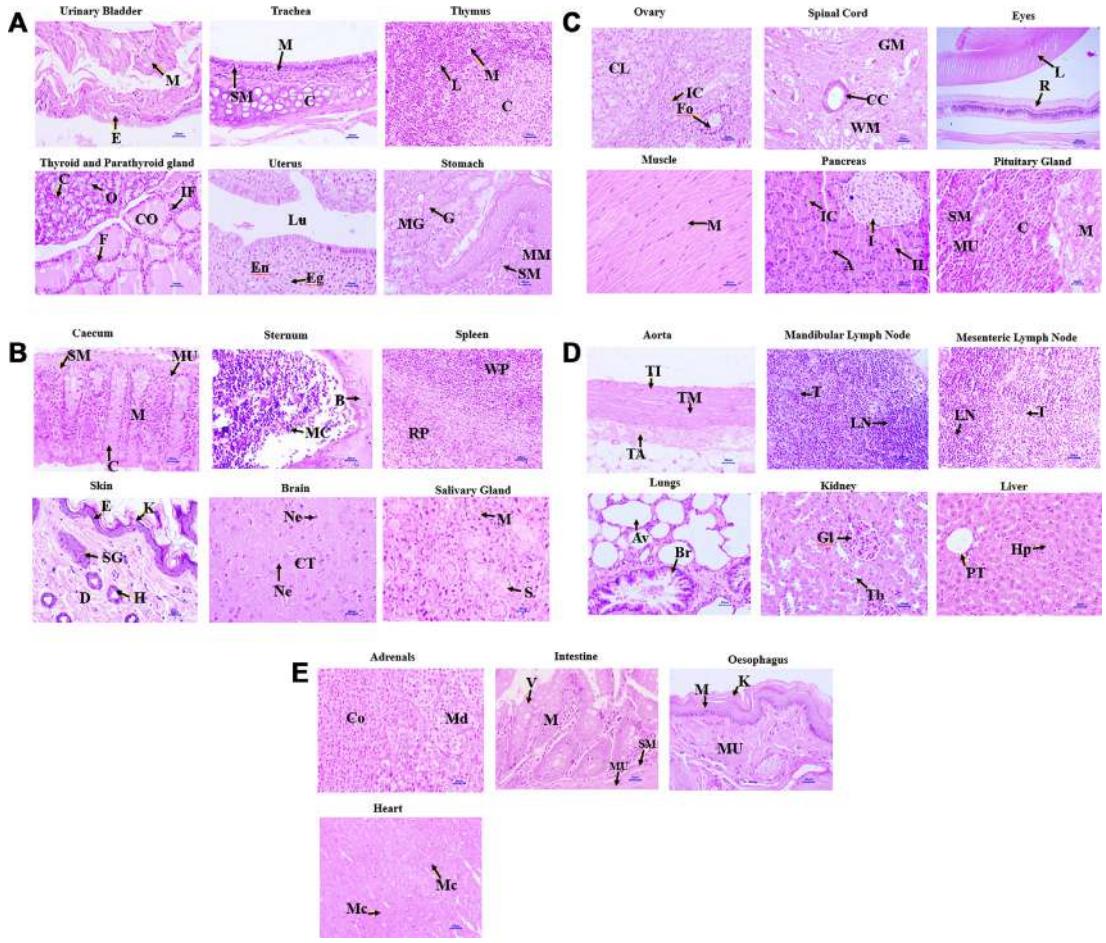

Fig. 4. Histopathology Images of Female High dose group. **A**: High Dose Group Female (H and E staining): **Urinary Bladder:** Showing normal histology, epithelium (E) and muscle (M), **Trachea:** Showing normal histology, tracheal cartilage (C), mucosa (M) and submucosa (SM), **Thymus:** Showing normal histology, lymphocytes (L) at medulla (M) and cortex (C), **Thyroid and Parathyroid gland:** Showing normal histology, Chief cell (C), Oxyphil cell (O) at parathyroid gland and Follicular lamina filled with acidophilic colloid (CO), Follicular epithelium (F), Interfollicular cells (IF), **Uterus:** Showing normal histology, endometrium (En), endometrial glands (Eg), Lumen (Lu), **Stomach:** Showing normal histology, mucosa at muscular stomach (MM), mucosa at glandular stomach (MG), submucosa (SM), gland (G). **B**: High Dose Group Female (H and E staining): **Cecum:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU), **Sternum:** Showing normal histology, cells at bone marrow (MC), bone (B), **Spleen:** Showing normal histology, White Pulp (WP), Red Pulp (RP), **Skin:** Showing normal histology, Epithelium (E), sebaceous gland (SG), keratin (K), dermis (D), hair follicle (H), **Brain:** Showing normal histology, neurons (Ne) at cortex (CT), **Salivary gland:** Showing normal histology, mucous gland acini (M), serous gland acini (S). **C**: High Dose Group Female (H and E staining): **Ovary:** Showing normal histology, Follicle (Fo), Interstitial cells (IC) and Corpus luteum (CL), **Spinal Cord:** Showing normal histology, Central canal (CC), Gray matter (GM), White matter (WM), **Eyes:** Showing normal histology, Lens (L), Retina (R), Optic nerve (O), **Muscle:** Showing normal histology, myocyte (M) at muscle fiber, **Pancreas:** Showing normal histology, acini (A), interlobular duct (IL), intercalated duct (IC), Islets of Langerhans (I), **Pituitary Gland:** Showing normal histology, Mucosa (M), Crypt (C), Submucosa (SM), Muscularis (MU). **D**: High Dose Group Female (H and E staining): **Aorta:** Showing normal histology, Tunic adventitia (TA), Tunica media (TM), Tunica interna (TI), **Mandibular Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Mesenteric Lymph Node:** Showing normal histology, Lymphoid nodule (LN), trabecula (T), **Lungs:** Showing normal histology, Bronchi (Br) and Alveoli (Av), **Kidney:** Showing normal histology, Glomerulus (Gl), Tubule (Tb), **Liver:** Showing normal histology, Portal triad (PT), Hepatocyte (Hp). **E**: High Dose Group Female (H and E staining): **Adrenals:** Showing normal histology, Cortex (Co) and Medulla (Md), **Intestine:** Showing normal histology, Mucosa (M), Villi (V), Submucosa (SM), Muscularis (MU), **Esophagus:** Showing normal histology, Mucosa (M), keratin layer (K), Muscularis (MU), **Heart:** Showing normal histology, Myocyte (Mc).

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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Declaration of competing interest

The authors have no conflicts of interest to declare.

References

[1] Ramakrishna BS, Venkataraman S, Mukhopadhya A. Tropical malabsorption. Postgrad Med J 2006;82:779–87. https://doi.org/10.1136/pgmj.2006.048579.
[2] Mandell Douglas, Bennett. Principles and practice of infectious diseases. 8th ed., vol. 39; 2014. p. 1298.
[3] Dash B. Diagnosis and treatment of diseases in Ayurveda (based on Ayurveda Saukhyam of Todarananda), vols. 1–5. New Delhi: Concept Publishing Company; 1984. p. 2578.
[4] Dastur JF. Everybody's guide to Ayurvedic medicine - a repertory of therapeutic prescriptions based on the indigenous system of India. Bombay: Taraporevala. Sons and Co.; 1960. p. 212.
[5] Mishra LC. Scientific basis for Ayurvedic therapies, vol. 22. CRC Press; 2010. p. 626. Reprint, 2010.
[6] Nadkarni AK. Indian Materia Medica. Bombay, India: Popular Book Depot; 1976. p. 1.
[7] Verma HK. Comprehensive book of Ayurvedic medicine for general practitioners with annonated key references (based on modern diagnosis and Ayurvedic treatment), vol. 1. New Delhi: Kalyani Publishers; 1991. p. 196.
[8] Shamkuwar P, Shahi S. Antimotility and antisecretory effect of Kutajarishta: an ayurvedic antidiarrhoeal formulation. Corpus ID: 39448812. 2012.
[9] Kumar Y, Singh BM, Gupta P. Clinical and metabolic markers based study of Swas Kasa Chintamani Rasa (An Ayurvedic herbo-metallic preparation) in childhood bronchial asthma (Tamak Swas). Int J Green Pharm 2014;8(1):37–44. https://doi.org/10.4103/0973-8258.126819.
[10] Jaisawal V, Shamal SN, Singh BM. Teratogenic effect of Swasa-Kasa-Chintamani-Rasa (SKCR) in animal model. World J Pharm Res 2015;4(4):1032–45.
[11] Vaidya Pandit Hariprasanna, Rasayogasagar. Vol-II, Chaukhamba Krishnadas Academy, Varanasi, Reprint, 1998. p. 466.
[12] Islam T, Hussain MS, Amin MN, Tuhin AM. Evaluation of psychopharmacological and neurosafety profile of SwasKas Chintamani Ras (SKC) in Swiss-Webster mice. Avicenna. J Phytomed. 2018;8(1):85–95.
[13] Guidance documents on repeated dose oral toxicity study in rodents. Organization for Economic Co-operation and Development (OECD) No. 452; 2018.
[14] Tatke P. Piperine enhances the bioavailability of secnidazole in rats. MOJ Bioequiv Bioavailab 2017;3(3). https://doi.org/10.15406/mojbb.2017.03.00037.
[15] Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry. Clin Chem Lab Med 1999;37(11/12):1136. 652.
[16] Tietz NW. Clinical guide to laboratory tests. 3rd ed. Philadelphia (PA): WB Saunders and Company; 1995. p. 76.
[17] Wilkinson JH, Boutwell JH, Winsten S. Evaluation of a new system for the kinetic measurement of serum alkaline phosphatase. Clin Chem 1969;15:487–95.
[18] Pearlman FC, Lee RT. Detection and measurement of total bilirubin in serum, with use of surfactants as solubilizing agents. Clin Chem 1974;20(4):447–53.
[19] Tietz NW, editor. Textbook of clinical chemistry. Philadelphia (PA): WB Saunders; 1986. p. 579. https://doi.org/10.1016/0307-4412(86)90182-2.
[20] Doumas BT, Arends RL, Pinto PC. Standard methods of clinical chemistry, vol. VII. Chicago: Academic Press; 1972. p. 175–89.
[21] Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest 1965;17:381–7. https://doi.org/10.3109/00365516509077065.
[22] Talke H, Schubert GE. Enzymatic urea determination in the blood and serum in the Warburg optical test. Klin Wochenschr 1965;43:174–5. https://doi.org/10.1007/BF01484513.
[23] Moorehead WR, Biggs HG. 2-Amino-2-methyl-1-propanol as the alkalizing agent in an improved continuous-flow cresolphthalein complexone procedure for calcium in serum. Clin Chem 1974;20:1458–60.
[24] Pennock CA, Murphy D, Sellers J, Longdon KJ. A comparison of autoanalyser methods for the estimation of glucose in blood. Clin Chim Acta 1973;48:193–201. https://doi.org/10.1016/0009-8981(73)90365-3.
[25] Roeschlau P, Bernt E, Gruber WA. Enzymatic determination of total cholesterol in serum. J Clin Chem Clin Biochem 1974;12:226.
[26] Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28:2077–80.
[27] Thakur KS, Vahalia MK, Jonnalagadda VG, Rashmi K, Nadkarni SD, Gudi RV. Evaluation of structural, chemical characterisation and safety studies of Samagandhak Kajjali, an Indian traditional ayurvedic drug. J Pharmacogn Phytochem 2014;2:57–67. Corpus ID: 56213446.
[28] Sharma MK, Kumar M, Kumar A. Ocimum sanctum leaf extract provides protection against mercury induced toxicity in Swiss albino mice. Indian J Exp Biol 2002;40:1079–82.
[29] Samudralwar DL, Garg AN. Minor and trace elemental determination in the Indian herbal and other medicinal preparations. Biol Trace Elem Res 1996;54:113–21. https://doi.org/10.1007/BF02786258.
[30] Timbrell JA. Principles of biochemical toxicology. London: Taylor and Francis Limited; 1982. https://doi.org/10.3109/9781420007084.
[31] El-Shenawy SM, Hassan NS. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. Pharmacol Rep 2008;60:199–208.
[32] Kumar G, Srivastava A, Sharma K, Kumar Gupta Y. Safety evaluation of mercury based Ayurvedic formulation (Sidh Makardhwaj) on brain cerebrum, liver & kidney in rats. Indian J Med Res 2014;139:610–8.