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Original Article
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Assessment of Chronic Toxicity of an Ayurvedic Herbo-Metallic Formulation Rasaraj Rasa in Wistar Rats

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Objectives: This study aimed to assess the adverse effects of Rasaraj Rasa tablets after repeated oral administration for 180 days in Wistar rats.
Methods: Wistar rats were divided into five groups, of which three were treated with 54, 162, and 270 mg/kg body weight of Rasaraj Rasa, respectively, which correspond to one, three, and five times the proposed human therapeutic dose, for 180 days consecutively. The fifth group (satellite) also received 270 mg/kg body weight of Rasaraj Rasa for 180 days. Body weight and food intake were measured weekly. At the end of the study, all rats were sacrificed, and their blood, serum, and organs were collected and examined using hematology, serum biochemistry, gross pathology, and histopathology tests. In contrast, the satellite group was kept for 4 weeks after treatment.
Results: No significant treatment-related toxicological findings were observed in the clinical features, body weight, laboratory findings, and pathological findings of the high-dose treated groups, when compared to those of the control group.
Conclusion: The no-observed-adverse-effect-level for Rasaraj Rasa in Wistar rats is set at 270 mg/kg body weight.

Keywords: ayurveda, wistar rats, chronic toxicity study, rasaraj rasa, herbo-metallic, noael

INTRODUCTION

The Ayurveda system continues to be built with a solid philosophical and experimental foundation. It is a biological science that emphasizes customized treatment and an integrated approach to health. It is also an all-encompassing medical system that includes mental, emotional, philosophical, ethical and spiritual wellness [1]. "Rasa Shastra" is a branch of Ayurveda that uses herbometallic remedies, which have played a significant role in treating chronic disorders in Indian and Chinese medicines [2, 3]. These medicinal compositions contain mercury, gold, iron, copper, zinc, and other metals. Herbometallic preparations have long been used due to their medicinal potential [4]. The toxic effects of heavy metals, such as mercury, lead, cadmium, and arsenic, have been documented. Mercury, particularly mercury salts and organic mercury (methyl mercury), has been linked to neurological problems and dementia [5, 6]. Thus, modern therapeutic professionals do not support treatment with mercury. Nonetheless, traditional medicine practitioners favor the utilization of processed metals. Metals are incinerated to remove their toxicity. Additionally, these formulations include organic plant extract components to make them biocompatible [7]. Specific herbs are used in processing metal preparations, a common trend among traditional medicine systems worldwide [8]. These herbs reportedly remove impurities and nullify toxic effects of metal fractions, further enhancing tissue-specific drug delivery and therapeutic potential [9]. The safety of Ayurvedic herbometallic formulations has

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been questioned in Western publications over the past ten years [10]. It became necessary for researchers to validate the safety of herbometallic formulations under the auspices of contemporary scientific principles and guidelines to maintain the trust of patients who are utilizing Ayurvedic formulations.

Rasaraj Rasa tablet is an Ayurvedic generic herbometallic formulation mentioned in Bhaishajya Ratnavali (Vatavyadhi) 26/204-208, an approved text under the first schedule of Drugs & Cosmetics Act, 1940 and Rules, 1945. Each tablet contains the following ingredients: Rasasindoor (34.483 mg), Suvarna (svarna) Bhasma (4.310 mg), Abhraka Bhasma (8.621 mg), Loha Bhasma (2.155 mg), Rajata Bhasma (2.155 mg), Vanga Bhasma (2.155 mg), Ashvagandha (*Withania somnifera*) (4.310 mg), Lavanga (*Syzygium aromaticum*) (2.155 mg), Jatipatri (*Myristica fragrans*) (2.155 mg), processed in Kumari (*Aloe barbadensis*) leaf swaras (juice) and Kakamachi (*Solanum nigrum*) whole-plant kwath (decoction; quantity sufficient [q.s.]).

Rasaraj Rasa is often indicated for long-term treatment of hemiplegia, paralysis, and lockjaw [11]. There are no reports on the toxicity of Rasaraj Rasa in animals, despite individual components being tested for toxicity.

This study aimed to present the combined toxicological effects of Rasaraj Rasa's many ingredients. Wistar rats were treated long-term with oral Rasaraj Rasa. All experiments were designed in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines [12].

MATERIALS AND METHODS

1. Animals

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC protocol approval number- SDARF/CT/2016/02). The study was performed at the lab animal house facility, Shree Dhootapapeshwar Ayurvedic Research Foundation (SDARF; Panvel, India) (CPCSEA Reg No: 136/PO/RcBi/S/99/CPCSEA). In total, 140 in-house-bred Wistar rats (age, 8-10 weeks) were used were acclimatized for 7 days, and housed in controlled environmental conditions within sterile polypropylene cages with a 12:12-hour light-dark cycle and a room temperature and humidity of 23 ± 1℃ and 55 ± 5%, respectively. All animals were given complete pelleted feed (Amrut, Prashanth Enterprises Ltd., Pune) and purified drinking water (Kent RO system) *ad libitum*.

2. Chemicals

Rasaraj Rasa tablets were procured from Shree Dhootapapeshwar Limited (SDL), Panvel, India. Carboxymethyl cellulose (CMC) and isoflurane USP solutions were procured from Loba Chemie Pvt. Ltd., Mumbai, and Raman & Weil Pvt. Ltd, Mumbai, respectively.

3. Experimental design

The rats were randomly divided into five groups as described in Table 1.

The administered doses were calculated using the approved human dose and a conversion factor of 0.018 [13]. The therapeutic dose (TD) of Rasaraj Rasa in humans is one or two 150-mg tablets twice a day. For the 180-day toxicity study, the highest TD was calculated to be 5 TD [14]. The highest dose was based on an acute oral toxicity study of Rasaraj Rasa in Wistar rats. A limit dose of 270 mg/kg body weight, which is equivalent to 5 TD, did not cause any mortality or toxicity-related sign during an observation period of 14 days. This suggests that Rasaraj Rasa is nontoxic (unpublished data).

4. Dose preparation

Rasaraj Rasa tablets were made in fine powder using mortar and pestle. The test item was measured and formulated with fresh CMC (1% w/v) on each day prior to dosing. The immiscible suspension was then administered orally to animals with oral gavage once a day for 180 days. Control animals were offered with 1% w/v of CMC for 180 days. The dose volume administered to each animal was calculated considering a standard of 10 mL/kg body weight.

Table 1. Experimental outline

Group no.	Groups	Dose of Rasaraj Rasa (mg/kg) B.W.	No. of males	No. of females
I	Control (Vehicle alone)	1% CMC	15	15
II	Low dose group (TD)	54	15	15
III	Mid dose group (3 TD)	162	15	15
IV	High dose group (5 TD)	270	15	15
V	Satellite group (5 TD)	270	10	10

5. Chronic toxicity study

The experiment was designed according to Organization for Economic Cooperation and Development (OECD) guideline 452. All animals were treated for 180 days. All dosages are mentioned in Table 1. The satellite group was kept for 4 weeks after completion of 180 days [12].

6. Observations

The following parameters were observed thrice (before, during, and after dosing): general appearance, body position and posture, autonomic nervous system function, motor coordination, reaction to physical handling and environmental stimulation, neurological signs (e.g., tremor, convulsion), abnormal behavior (e.g. abnormal vocalization) and aggression, lacrimation, salivation, and gait pattern.

7. Mortality and clinical signs

Throughout the study, morbidity and mortality were checked twice. All signs of poor health, behavioral changes, or adverse reactions were recorded once daily. Detailed physical examinations were performed weekly.

8. Body weight

Body weights were recorded once before initiation of dosing and weekly thereafter until the end of the experiment. Additionally, overnight fasted body weights were recorded before autopsy.

9. Clinical pathology

After completion of treatment, blood samples were collected from overnight fasted animals through the retro-orbital plexus and placed in two separate vials, to be used hematological (EDTA used as an anticoagulant) and biochemical analyses. Blood samples were collected from the satellite group at 208 days after initiation of treatment to see any reversal of treatment-related findings. All animals were anesthetized with isoflurane anesthesia (1-2 mL) prior to blood collection.

1. Hematological analysis

Freshly collected blood samples were placed in a fully automated cell counter – Beckman Coulter. Hematological parameters, such as hemoglobin concentration (HGB), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hematocrit level (HCT), were measured.

2. Serum biochemistry

The serum samples were separated after incubation of whole blood at 4-8℃ and were stored at –20℃ for further analysis. Biochemical analysis was performed by using a fully automated biochemistry analyzer – Randox Daytona Plus. Standardized diagnostic kits (Randox) were utilized for estimating the total bilirubin (mg/dL), direct bilirubin (mg/dL), indirect bilirubin (mg/dL), aspartate transaminase (AST/SGOT) (U/L), alanine transaminase (ALT/SGPT) (U/L), alkaline phosphatase (ALP) (u/L), total protein (g/dL), albumin (g/dL), globulin (g/dL), albumin-globulin ratio (mg/dL), creatinine (mg/dL), urea (mg/dL), uric acid (mg/dL), calcium (Ca) (mEq/L), phosphorus (P) (mEq/L), total cholesterol (mg/dL), triglyceride (mg/dL), and glucose (mg/dL) levels.

3. Gross pathology and histopathology

1) Methodology

All surviving animals belonging to different groups were euthanized using carbon dioxide asphyxiation before performing external examinations. Autopsies of different organs, including the brain, heart, liver, kidney, lungs, adrenal gland, spleen, testes, epididymis, ovary, and uterus, were also performed. The organs were collected, weighed, and processed for histopathology. Animals from the satellite group were sacrificed on the 208th day, with the same procedures conducted afterwards.

The fixed tissues were routinely processed and stained with hematoxylin and eosin (H&E). Initially, all the organs/tissues were examined macroscopically. All gross lesions and above-mentioned tissues were sectioned and H&E-stained. The slides were observed for any histopathological lesions. Lesions were classified according to severity as follows: 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%). The distribution of the lesions was recorded and categorized as focal, multifocal, or diffuse.

If significant changes were observed in the organs of the high-dose treatment group, the organs from all other treatment groups were analyzed. If no changes were observed, no further groups were analyzed for histopathological staining.

10. Statistical analysis

Data were expressed as means and standard deviations, and analyzed using Graph Pad Prism Software. Data were analyzed for dose-wise comparison. One-way ANOVA followed by Tukey's test was used to compare the groups.

RESULTS

1. General symptoms

In both sexes, there were no changes in general appearance, including skin coloration, fur, mucosa, gait pattern, sensorimotor responses to visual, acoustic, tactile, and painful stimuli (reactivity and sensitivity). Clinical symptoms, such as posture, position, motor coordination, reaction to physical and environmental stimulation, neurological findings (*e.g.*, tremor, convulsion, muscular contractions), abnormal behavior (abnormal vocalization) and aggression were also similar among the groups.

2. Mortality

No treatment-related mortality and clinical signs were noticed in the treated animals. Gross autopsy and histopathology did not reveal any lesions of toxicological importance.

3. Body weight

There were no observable significant changes in the body weight of male and female animals in all treated groups, compared with that of the control group (Table 2).

4. Feed and water consumption

There were no significant changes observed in food and water intake throughout the treatment period in all groups.

5. Clinical pathology

1. Hematology

There were no significant alterations observed among hematological parameters in all treated groups, as compared to those of the control group in both sexes. However, both increasing or decreasing trends were observed. Significant variations in females included a decrease in HCT in the mid-dose and satellite groups, compared to that of the low-dose group, and an increase in MCH in the mid-dose group, compared to that in the high-dose group.

All above variations were well within normal physiological limits; hence, they had no toxicological significance (Tables 3, 4).

2. Serum biochemistry

Serum biochemistry analysis did not reveal any significant treatment-associated alterations in parameters in all treated

Table 2. Effect of oral administration of Rasaraj Rasa on body weight in males and females

Groups	1st week	27th week
Body weight males [mean body weight (in g) ± SD]		
Control group	164.13 ± 7.98	510 ± 14.03
Low dose group (TD)	176.87 ± 14.18	523.07 ± 12.81
Mid dose group (3 TD)	165.6 ± 9.12	489.36 ± 39.58
High dose group (5 TD)	185.27 ± 8.7	492.4 ± 28.47
Satellite group (5 TD)	167.3 ± 17.76	498 ± 35.75
Body weight females [mean body weight (in g) ± SD]		
Control group	150.93 ± 13.99	302 ± 36.59
Low dose group (TD)	154.2 ± 15.37	305.73 ± 18.52
Mid dose group (3 TD)	147.33 ± 13.89	312.27 ± 24.11
High dose group (5 TD)	155.6 ± 11.66	301.67 ± 25.65
Satellite group (5 TD)	139.8 ± 15.83	296.8 ± 27.03

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

Table 3. Effect of Rasaraj Rasa on hematology parameters in male Wistar rats

Parameters	Control group	Low dose group (TD)	Mid dose group (3 TD)	High dose group (5 TD)	Satellite group (5 TD)
WBC ($\times 10^3/\mu L$)	10.76 ± 1.28	11.26 ± 1.44	10.36 ± 0.97	10.97 ± 1.04	11.73 ± 1.03
RBC ($\times 10^6/\mu L$)	7.78 ± 0.62	7.79 ± 0.53	7.87 ± 0.54	7.64 ± 0.76	7.37 ± 0.71
Hgb (g/dL)	14.82 ± 0.97	14.72 ± 1.08	15.34 ± 0.76	14.48 ± 0.99	14.63 ± 0.69
HCT (%)	43.63 ± 2.03	43.6 ± 1.76	44.74 ± 2.39	44.46 ± 2.85	44.36 ± 2.99
MCV (fl)	56.31 ± 3.71	56.26 ± 4.91	57.03 ± 4.25	58.81 ± 7.69	60.66 ± 6.73
MCH (pg)	19.17 ± 2.04	18.97 ± 1.83	19.6 ± 2	19.17 ± 2.6	20 ± 1.76

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

Table 4. Effect of Rasaraj Rasa on hematology parameters in female Wistar rats

Parameters	Control group	Low dose group (TD)	Mid dose group (3 TD)	High dose group (5 TD)	Satellite group (5 TD)
WBC ($\times 10^3/\mu L$)	11.51 ± 1.5	11.71 ± 1.68	11.34 ± 1.0	12.77 ± 3.12	12.7 ± 1.77
RBC ($\times 10^6/\mu L$)	7.9 ± 0.33	7.82 ± 0.41	7.51 ± 0.32	7.93 ± 0.47	7.76 ± 0.45
Hgb (g/dL)	15.15 ± 0.87	15.26 ± 0.79	15.09 ± 0.6	14.91 ± 0.55	14.52 ± 0.6
HCT (%)	45 ± 1.27	45.68 ± 3.06	43.14 ± 1.7$^{\#}$	44.88 ± 2.42	42.93 ± 2.61$^{\#}$
MCV (fl)	57.09 ± 3.02	58.59 ± 5.31	57.51 ± 3.15	56.83 ± 4.88	55.41 ± 2.94
MCH (pg)	19.22 ± 1.508	19.56 ± 1.24	20.1 ± 0.91$^{\#}$	18.85 ± 1.042	18.78 ± 1.01

Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA followed by Tukey's test for comparison. Level of significance $^{\#}p < 0.01$.

groups when compared with the control group in both sexes. However, few biologically significant variations were observed in some parameters. Those variations in males included an increase in ALP ($p < 0.01$) in the high-dose group, increase in serum urea ($p < 0.05$) levels in the high-dose and satellite groups, and a significant decrease in globulin ($p < 0.05$) in the mid-dose group and in glucose ($p < 0.01$) in the satellite group, when compared with the control group. Variations in females included a significant increase in alkaline phosphate ($p < 0.05$) in the high-dose group and a significant decrease in glucose ($p < 0.01$) in the satellite group, when compared with the control group.

All above variations were well within normal physiological limits; hence, they had no toxicological significance (Tables 5, 6).

6. Histopathology

1. Macroscopic findings

Gross pathological observations in both control and treated groups did not reveal any lesions of toxicological significance.

1) External

External examination of all groups did not show any significant lesions.

2) Internal

The visceral examination of all groups did not show any significant lesions.

2. Microscopic findings

Treatment-related microscopic lesions were not observed among all groups. Microscopic examination showed focal to multifocal minimal lymphocytic infiltration in the liver, focal and multifocal minimal to mild lymphocytic and tubular mineralization in the kidney, multifocal mild lymphocytic infiltration and minimal alveolar histiocytosis in the lungs, unilateral accessory adrenocortical tissue, and multifocal minimal to mild increased extra-medullary hematopoiesis in the spleen. All these lesions were few and observed in both control and high-dose groups.

All observed findings were common in terms of age and strains used in the study. They were considered as spontaneous or incidental, owing to similar frequencies of occurrence between the control and high-dose groups.

Except for spontaneous lesions, there were no treatment-related lesions of toxicological significance observed in different tissues from the high-dose group (Figs. 1, 2).

Therefore, the organs of the low- and mid-dose groups were not stained. Histopathological slides of the organs were ob-

Table 5. Effect of Rasaraj Rasa on serum biochemistry parameters in male Wistar rats

Parameters	Control group	Low dose group (TD)	Mid dose group (3 TD)	High dose group (5 TD)	Satellite group (5 TD)
S. Bilirubin (Total) (mg/dL)	0.36 ± 0.1	0.36 ± 0.09	0.34 ± 0.11	0.32 ± 0.1	0.28 ± 0.04
S. Bilirubin (Direct) (mg/dL)	0.22 ± 0.1	0.24 ± 0.07	0.24 ± 0.11	0.22 ± 0.1	0.18 ± 0.04
S. Bilirubin (indirect) (mg/dL)	0.14 ± 0.05	0.12 ± 0.04	0.1 ± 0.03	0.1 ± 0.0001	0.1 ± 0.0001
S.G.O.T. (U/L)	120.17 ± 16.2	95.92 ± 12.25	119.2 ± 14.61	129.37 ± 9.85	118.18 ± 16.89
S.G.P.T (U/L)	54.16 ± 7.44	51.19 ± 6.26	57.66 ± 7.15	61.54 ± 14.51	63.44 ± 7.68
Alkaline Phosphatase (U/L)	348.32 ± 49.72	303.15 ± 49.06	397.99 ± 47.19	421.54 ± 48.59$^{\#}$	383.39 ± 59.44
Total Proteins (g/dL)	8.3 ± 1.66	7.85 ± 1.35	6.51 ± 1.7	7.3 ± 1.43	8.19 ± 0.4
Albumin (g/dL)	3.62 ± 0.69	3.63 ± 0.54	3.21 ± 0.76	3.52 ± 0.61	3.94 ± 0.16
Globulin (g/dL)	4.68 ± 1.01	4.22 ± 0.87	3.37 ± 0.89*	3.78 ± 0.86	4.25 ± 0.45
A/G Ratio (mg/dL)	0.79 ± 0.07	0.87 ± 0.09	0.95 ± 0.1	0.96 ± 0.13	0.95 ± 0.14
Creatinine (mg/dL)	0.8 ± 0.16	0.76 ± 0.14	0.65 ± 0.15	0.73 ± 0.12	0.77 ± 0.04
Serum Urea (mg/dL)	29.35 ±5.47	27.98 ±5.92	23.73 ± 4.99	35.17 ± 6.15*	36.33 ± 3.3*
Uric Acid (mg/dL)	2.09 ± 0.88	2.24 ± 1.04	2.04 ± 0.75	1.63 ± 0.37	1.56 ± 0.32
Calcium (mg/dL)	10.73 ± 2.32	9.99 ± 1.87	9.04 ± 1.33	10.08 ± 0.99	10.86 ± 0.51
Phosphorous (mg/dL)	5.25 ± 0.49	5.12 ± 0.38	4.73 ± 0.39	5.13 ± 0.6	5.33 ± 0.31
Total Cholesterol (mg/dL)	52.02 ± 11.55	52.61 ± 15.05	43.7 ± 9.15	49.37 ± 10.14	55.19 ± 8.9
Triglycerides (mg/dL)	98.35 ± 9.81	94.45 ± 9.21	102.12 ± 19.97	102.18 ± 23.07	101.25 ± 24.51
Glucose (mg/dL)	98.68 ± 7.67	89.91 ± 5.56	105.78 ± 8.49	93.33 ± 7.01	87.58 ± 10.69$^{\#}$

Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA followed by Tukey's test for comparison. Level of significance *$p < 0.05$; $^{\#}p < 0.01$.

Table 6. Effect of Rasaraj Rasa on serum biochemistry parameters in female Wistar rats

Parameters	Control group	Low dose group (TD)	Mid dose group (3 TD)	High dose group (5 TD)	Satellite group (5 TD)
S. Bilirubin (Total) (mg/dL)	0.37 ± 0.1	0.3 ± 0.1	0.34 ± 0.091	0.31 ± 0.07	0.33 ± 0.11
S. Bilirubin (Direct) (mg/dL)	0.25 ± 0.1	0.19 ± 0.1	0.23 ± 0.1	0.21 ± 0.07	0.23 ± 0.11
S. Bilirubin (indirect) (mg/dL)	0.11 ± 0.04	0.11 ± 0.04	0.11 ± 0.02	0.1 ± 0.0001	0.1 ± 0.00001
S.G.O.T. (U/L)	113.1 ± 15.26	97.25 ± 12.58	114.03 ± 12.56	125.54 ± 12.42	121.77 ± 13.81
S.G.P.T (U/L)	57.21 ± 16.65	49.55 ± 9.45	54.88 ± 9.69	57.58 ± 10.71	60.56 ± 8.38
Alkaline Phosphatase (U/L)	335.82 ± 57.15	295.2 ±59.26	386.58 ± 48.48	402.12 ± 57.46*	368.9 ± 74.7
Total Proteins (g/dL)	7.87 ± 2.45	7.72 ± 1.83	7.47 ± 2.04	8.06 ± 1.37	8.07 ± 1.14
Albumin (g/dL)	3.86 ± 1.17	3.94 ± 0.85	3.87 ± 1.03	4.2 ± 0.64	4.12 ± 0.51
Globulin (g/dL)	4.01 ± 1.35	3.78 ± 1.03	3.61 ± 1.05	3.86 ± 0.83	3.95 ± 0.72
A/G Ratio (mg/dL)	0.99 ± 0.14	1.073 ± 0.19	1.11 ± 0.22	1.1 ± 0.19	1.07 ± 0.16
Creatinine (mg/dL)	0.76 ± 0.23	0.73 ± 0.15	0.74 ± 0.19	0.72 ± 0.12	0.69 ± 0.07
Serum Urea (mg/dL)	31.82 ± 10.14	26.77 ± 5.28	29.83 ± 9.58	33.68 ± 6.4	34.19 ± 5.75
Uric Acid (mg/dL)	1.58 ± 0.72	2.08 ± 0.84	1.33 ± 0.51	1.49 ± 0.54	1.62 ± 0.57
Calcium (mg/dL)	10.13 ± 2.87	9.15 ± 3.01	9.67 ± 1.71	10.51 ± 1.46	10.42 ± 1.19
Phosphorous (mg/dL)	4.87 ± 0.41	4.44 ± 0.36	4.78 ± 0.48	5.03 ± 0.51	5.06 ± 0.78
Total Cholesterol (mg/dL)	58.6 ± 20.22	63.38 ± 14.84	60.5 ± 14.98	65 ± 16.55	57.86 ± 13.94
Triglycerides (mg/dL)	95.07 ± 12.001	90.32 ± 6.62	100.94 ± 16.4	105.18 ± 13.77	102.19 ± 30.61
Glucose (mg/dL)	99.67 ± 6.68	103.71 ± 8.39	101.84 ± 8.15	96.44 ± 7.53	81.84 ± 8.11$^{\#}$

Values are expressed as mean ± SD; n = 6; Data analyzed by One-way ANOVA followed by Tukey's test for comparison. Level of significance *$p < 0.05$; $^{\#}p < 0.01$.

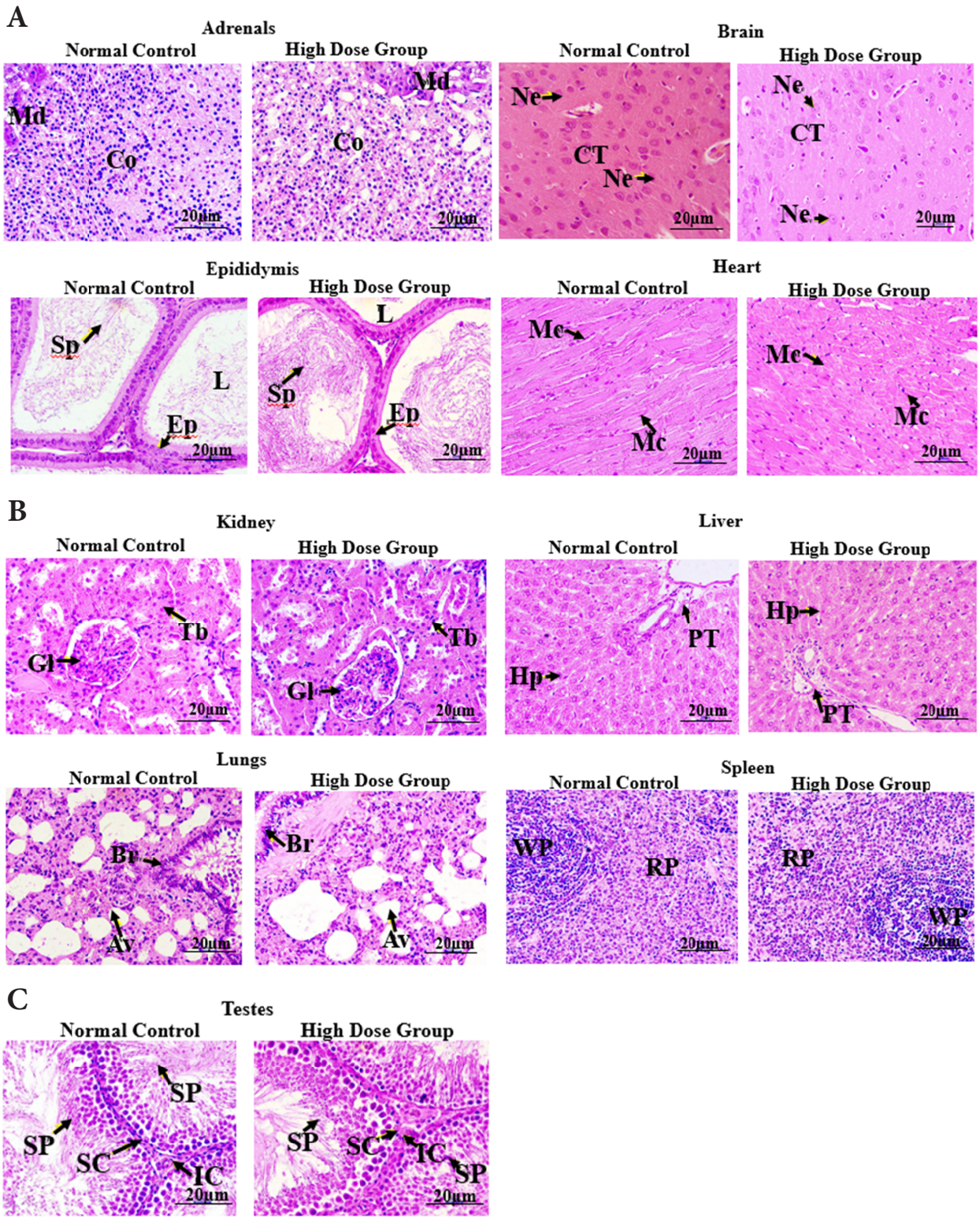

Figure 1. Histopathology images of male normal control group & high dose group. Normal control & high dose group male (H&E staining). (A) Adrenals: showing normal histology, cortex (Co) and medulla (Md). Brain: showing normal histology, neurons (Ne), cortex (CT). Epididymis: showing normal histology, sperm (Sp), luminal (L), epithelium (Ep). Heart: showing normal histology, myocyte (Mc). (B) Kidney: showing normal histology, glomerulus (Gl), tubule (Tb). Liver: showing normal histology, portal triad (PT), hepatocyte (Hp). Lungs: showing normal histology, bronchi (Br) and alveoli (Av). Spleen: showing normal histology, white pulp (WP), red pulp (RP). (C) Testes: showing normal histology of seminiferous tubule, spermatid (Sp), interstitial cells (IC), sertoli cell (SC).

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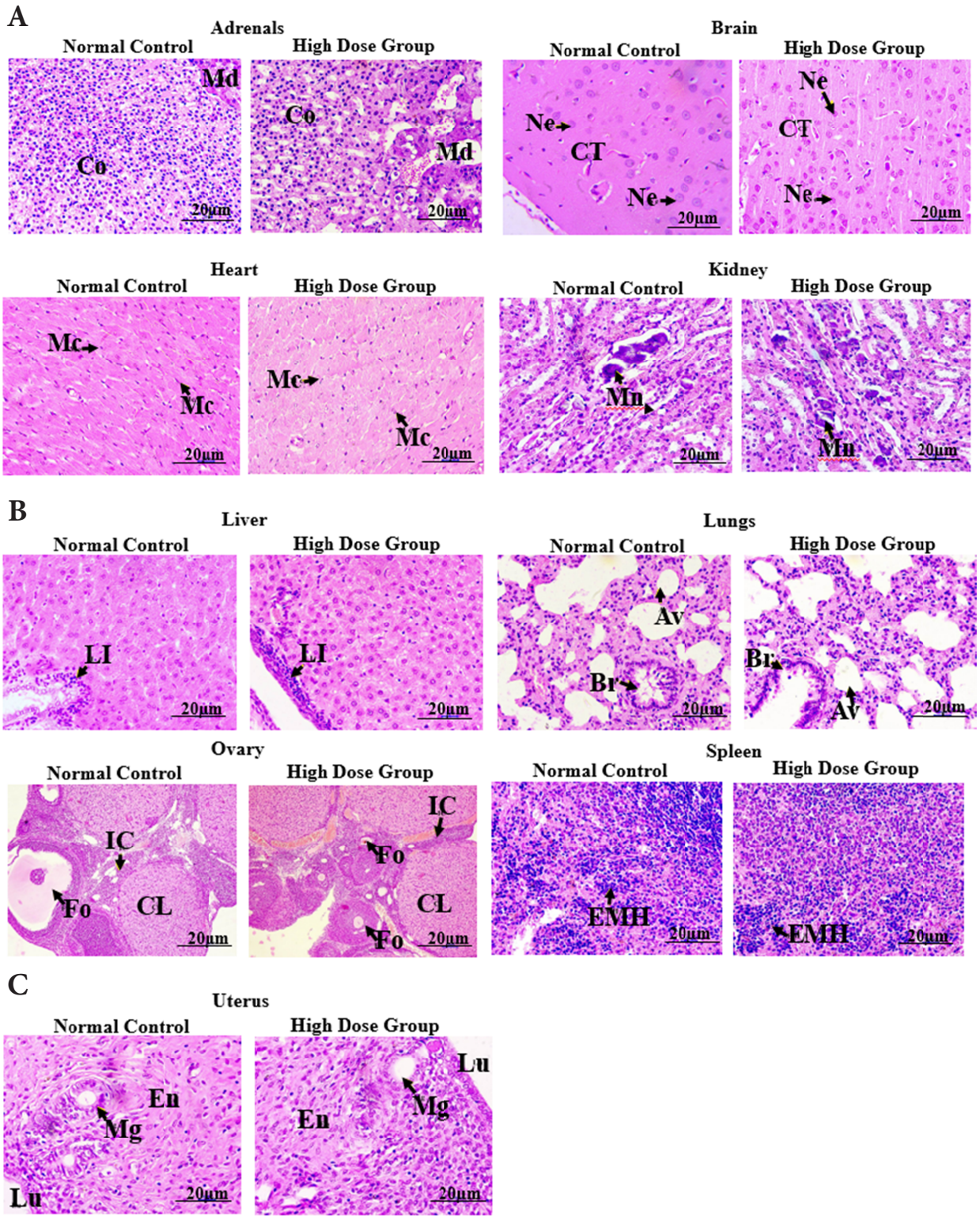

Figure 2. Histopathology images of female normal control group & high dose group. Normal control & high dose group female (H&E staining). (A) Adrenals: showing normal histology, cortex (Co) and medulla (Md). Brain: showing normal histology, neurons (Ne), cortex (CT). Heart: showing normal histology, myocyte (Mc). Kidney: showing spontaneous/incidental mineralization in tubules (Mn). (B) Liver: showing normal histology, spontaneous/incidental hepatocellular lymphocytic infiltration (LI). Lungs: showing normal histology, bronchi (Br), alveoli (Av). Ovary: showing normal histology, follicle (Fo), Interstitial cells (IC) and corpus luteum (CL). Spleen: showing spontaneous/incidental extramedullary hematopoiesis in red pulp (EMH). (C) Uterus: showing normal histology, endometrium (En), myometrial glands (Mg), lumen (Lu).

served microscopically, with images taken under 400× magnification.

DISCUSSION

Rasasindoor, Abhraka Bhasma, Suvarna Bhasma, Loha Bhasma are the major components of Rasaraj Rasa. Rasasindoor is prepared by mixing purified mercury and sulfur. The purification process renders mercury nontoxic [11]. Gokarn et al. [15] reported that Rasasindoor is safe to use up to 10 TD (equivalent to 450 mg/kg body weight) in Wistar rats after conducting a 90-day repeated-oral dose toxicity study.

Loha Bhasma was found safe up to 5 TD when orally administered to rats [16]. Like-wise *Withania somnifera* [17], Vanga Bhasma [18] and Suvarna Bhasma [19] are reported to be safe in repeated oral dose administration.

Toxicity studies of Rasaraj Rasa in lab animals help to find out the highest non-toxic dose (tolerated dose), as well as assess any toxicity-related alterations in hematology, serum biochemistry and histology.

Changes in body weight is an important factor in monitoring the health of an animal. Loss of body weight is usually the first sign indicating the onset of an adverse effect. Doses which cause at least a 10% loss in body weight are considered toxic [20].

The body weight changes (Table 2) in treated groups did not differ significantly as compared to normal control group, which is an indicative of the absence of toxic effect of Rasaraj Rasa during chronic administration in rats.

No significant changes in hematology parameters were observed in the male treated groups (Table 3). All changes in hematological parameter levels after treatment with Rasaraj Rasa were within physiological limits and do not carry any toxicological significance.

The increase in ALP levels above the physiological limit is indicative of liver damage; however, serum ALP levels in the high dose group were within physiological limits, and hence do not carry toxicological significance (Tables 5, 6).

The rate of occurrence of microscopic lesions in the high-dose group was low, which compared well with that of the control group. Such lesions usually develop in the laboratory to a certain extent, and thus are considered as spontaneous or incidental. Similar lesions have been reported in previous toxicological studies [21-24]. All these results confirm the non-toxic nature of Rasaraj Rasa at various concentrations.

Lack of sufficient toxicological evidence is a significant hurdle in herbometallic research. Patients usually self-administer the herbal medications without proper guidance [25]. Inadequate scientific research on safety aspects along with inappropriate dosages is a major problem [26]. Our study results aim to attenuate this problem.

CONCLUSION

Wistar rats were able to tolerate repeated-dose administration of 270 mg/kg (equivalent to 5 TD) of Rasaraj Rasa for a consecutive period of 180 days. There were no adverse alterations in clinical signs, body and organ weight, consumption, and hematological, serum biochemical, gross pathological, and histological parameters. Thus, the no-observed adverse-effect level for test formulations of Rasaraj Rasa in Wistar rats could be set at 5 TD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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