Original Article

Chronic toxicity study of *Sameera Pannaga Rasa* in Charle's foster albino rats

Madhvi Sharma, Biswajyoti Patgiri[1], Mukesh B. Nariya[2], Shrirang Jamadagni[3], Prashant Bedarkar[4]

M.Pharm Scholar, [1]Professor, Department of Rasa Shastra and Bhaishajya Kalpana Including Drug Research, Institute of Teaching and Research in Ayurveda, Jamnagar, [2]Head, Pharmacology Laboratory, Institute of Teaching and Research in Ayurveda, Jamnagar, Gujarat, [3]Pharmacologist, Regional Ayurveda Institute for Fundamental Research Kothrud, Pune, Maharashtra, India, [4]Asso. Professor, Department of Rasa Shastra and Bhaishajya Kalpana Including Drug Research, Institute of Teaching and Research in Ayurveda, Jamnagar, Gujarat, India

Abstract

Introduction: *Sameera Pannaga Rasa* (SPR) is a *Kupi Pakwa Rasayana* (a mercurial–arsenical formulation of *Ayurveda* prepared by specific pharmaceutical-controlled, indirect heat treatment [sand bath] in glass bottle) that contains *Shodhita Parada* (processed mercury), *Shodhita Gandhaka* (processed sulfur), *Shodhita Haratala* (processed arsenic trisulfide), *Shodhita Somala* (processed arsenic oxide) and *Shodhita Manahshila* (process arsenic disulfide) in equal quantity as ingredients. *Parada*, *Haratala*, *Manahshila* and *Somala* are highly potent minerals which are included in the Drug and Cosmetic Act 1940 under Schedule E1 because of their toxic nature in crude form. **Materials and methods:** In the present study, SPR was evaluated for safety profile through its chronic toxicity study in Charle's foster albino rats. The test drug was made into suspension in vehicle (4 ml honey and 7 ml distilled water). The test drug was administered orally once a day for 90 consecutive days in the dose of 11.25 (therapeutic dose [TED]), 56.25 (5 times TED) and 112.25 mg/kg (10 times TED). Animals were sacrificed on 91st day and animals of recovery group were sacrificed on 121st day. Parameters such as hematological, serum biochemical, and histopathology of various organs were studied. **Results:** Test drug at a higher dose level and recovery study showed no toxic effect in albino rats during chronic toxicity study. **Conclusion:** SPR is found to have no toxic effect in albino rats during the repeated dose, oral, chronic toxicity study of 90 days, even at 10 times therapeutic equivalent dose (112.25 mg/kg) and even during recovery period of 1 month. It may be safety used at TED level.

Keywords: Chronic toxicity, *Gandhaka, Haratala, Manahshila, Parada, Sameera Pannaga Rasa, Somala*

Introduction

Herbomineral and metallic formulations are an important part of *Ayurveda* that are endorsed to be safe and efficacious when manufactured and used prudently. *Rasaushadhi* is an integral part of Ayurvedic therapeutics, which is effective in small doses, tasteless, and fast acting. *Sameera Pannaga Rasa* (SPR) is one of the important formulations mentioned under peculiar dosage form, *Kupi Pakwa Rasayana* (a mercurial–arsenical formulation of *Ayurveda* prepared by specific pharmaceutical-controlled, indirect heat treatment [sand bath] in glass bottle). Primarily, SPR has been mentioned in *Rasa Chandanshu* by the name of *Vata Pannaga*,[1] containing *Parada*, *Gandhaka*, *Haratala*, and *Somala* in equal parts, in which *Manahshila* has not been mentioned as a constituent. However, later on, in text *Ayurveda Aushadhi Guna Dharma Shastra*[2] by Gune, *Manahshila* was added to formulate composition of SPR which has been accepted by Ayurvedic Formulary of India.[3]

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Parada, *Haratala*, *Manahshila*, and *Somala* are among highly potent metal/mineral/metalloids which are included in the list of poisonous drugs by the Drug and Cosmetic Act 1940 under Schedule E(1)[4] because of their highly toxic nature in crude form. These poisonous materials are advised to use essentially after *Shodhana* process along with specific adjuvant or vehicles. SPR is mentioned for the management of *Sandhivata* (osteoarthritis), *Unmada* (insanity-mental disorder), *Kasa* (cough), *Jwara* (fever)[5] etc., It has been used extensively for *Tamaka Shwasa* (bronchial asthma),[6]

Address for correspondence: Miss. Madhvi Sharma, Department of Rasa Shastra and Bhaishajya Kalpana, Institute of Teaching and Research in Ayurveda, Jamnagar - 361 008, Gujarat, India.
E-mail: om.madhvisharma@gmail.com

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and it also shows good effects on *Amavata*.[7] SPR is a mercurial–arsenical formulation, so it is most essential to assure the safety of a drug through experimental studies.

Chronic toxicity is defined as adverse effects occurring after repeated or continuous administration of a test sample for a major part of the life span. The objective of a chronic toxicity study is to determine the effects of a substance following prolonged and repeated exposure.[8]

It can manifest as direct lethality but more commonly refers to sublethal end points, such as decreased growth, reduced reproduction, or behavioral changes. Considering all the characters of an individual content (mercurial–arsenicals), the formulation of SPR was tested to evaluate its safety profile through chronic repeated dose and toxicity in the current study.

Materials and Methods

Procurement of raw material

Parada, *Gandhaka*, *Haratala*, *Somala* and *Manahshila* were procured from the Institutional Pharmacy, while fresh leaves of *Tulsi* (*Ocimum sanctum* Linn.) were collected from the periphery of local district and authenticated by Pharmacognosy Laboratory of the Institute.

Preparation of test drug

Pharmaceutical manufacturing of SPR was carried out at the Department of Rasa Shastra and Bhaishajya Kalpana, IPGT and RA, Gujarat Ayurved University. *Shodhana* of *Parada*,[9] *Gandhaka*,[10] *Haratala*,[11] *Somala*[12] and *Manahshila*[13] were carried out as per classical references. SPR was prepared by following *Kupi Pakwa* method.[2,3,14] Equal quantity of *Shuddha Parada* and *Shuddha Gandhaka* was taken in edge runner and *Mardana* (dry trituration) was carried out till it became *Nishchandra* (lusterless) *Kajjali.* Powder of *Shuddha Haratala* (As_2S_3) and powder of *Shuddha Manahshila* (As_2S_2) were added one by one subsequently and triturated till it attains a homogenous mixture. Then, *Shuddha Somala* (As_2O_3) was added to it and triturated well to get a homogeneous mixture. *Sameera Pannaga Kajjali* was given three *Bhavana* (levigation/wet trituration) with *Tulsi Patra Swarasa* (juice of *O. sanctum* Linn. leaves). A clean *Kacha Kupi* (amber-colored narrow mouth, vertical glass bottle, cylindrical body, base and conical neck) with a volume of 750 ml was taken and mud (Fuller's earth) smeared cotton cloth pieces were wrapped over *Kupi* one by one for 7 times (7 coats of) uniformly and consecutively applied only after complete drying of the former layer. Prepared *Kajjali* was filled in seven-layered *Kacha Kupi* up to its two-third capacity and was properly, carefully placed in a vertical electric muffle furnace (VEMF). *Kajjali* containing *Kacha Kupi* was heated gradually in the VEMF at three different temperatures, i.e. *Mridu Agni* (mild heat), *Madhaym Agni* (moderate heat) and *Tivra Agni* (high heat), for a certain period. During the preparation of SPR, variation in heating temperature was observed to confirm the melting and boiling stage of *Kajjali*. *Shita Shalaka* (cold iron rod) was inserted into the neck of *Kupi* whenever necessary. Red hot iron rod (*Tapta Shalaka*) was inserted into the neck of *Kupi*, to clear deposited undesired materials inside the mouth of the *Kupi*. After 37.35 h of active heating, with a maximum temperature of 520°C, VEMF was switched off and *Kupi* was kept for self-cooling. Once *Kupi* was cooled, it was taken out and cleaned carefully. *Kupi* was broken in the middle to separate the lower and upper halves. For this, kerosene-dipped thread was wrapped on *Kupi* and ignited; then, wet cloth was wrapped immediately on *Kupi* for breaking. After breaking of *Kupi*, SPR deposited at either place, i.e. *Talastha* (bottom of the glass bottle) and *Kanthastha* (neck of the glass bottle), was procured by careful scraping and gentle tapping. The product was scraped, collected, and stored in an airtight container. In the present toxicity study, *Talastha* SPR (product obtained bottom of the glass bottle) was used.

Experimental animals

Charle's foster strain albino rats of either sex weighing 180 ± 220 g were used for the experimentation. The rats were procured from the animal house attached to the Pharmacology Laboratory of IPGT and RA, Jamnagar. The animals were exposed to day and night cycle under ideal laboratory conditions in terms of ambient temperature (22°C ± 3°C) and relative humidity (50%–70%). VRK brand rat pellet feed supplied by Pranav Agro Ltd. was provided to animals throughout the study period. Drinking water was given *ad libitum* in polypropylene bottles with a stainless steel sipper tube. The experiments were carried out after approval of Institutional Animal Ethics Committee (IAEC/21/2016/20) in conformity with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi.

Dose fixation and schedule

The therapeutic dose (TED) of SPR is 125 mg. Dose of the test drug was calculated by extrapolating the human TED to rat, based on body surface area ratio (conversion factor 0.018 for rat) by referring to the table of Paget and Barnes[15] which was found to be 11.25 mg/kg of rat. The test drug was made into fine suspension in vehicle (4 ml honey) with 7 ml distilled water. The test drug was administrated orally using cannula at different dose levels to respective groups in between 8:00 am and 9:30 am daily.

Chronic toxicity

The study was carried out by following prevailing guidelines (OECD 408,[16] General Guidelines for Safety/Toxicity Evaluation of Ayurvedic Formulations, Ministry of AYUSH, Government of India)[17] (90 days oral repeated dose toxicity study). Animals were selected and divided into five groups, each consisting of six rats comprising three males and three females. Animals in Group-I received vehicle (10 ml/kg honey orally) and served as vehicle control (VC) group, whereas Group-II to Group-V received SPR along with adjuvant (honey) at TED, 11.25 mg/kg orally), TED × 5 (56.25 mg/kg orally), TED × 10 (112.25 mg/kg orally), and TED ×10 recovery (112.25 mg/kg orally) groups, respectively.

The test drug was administered orally once a day for 90 consecutive days. Body weight of all animals was noted down before the commencement of the study and afterward every 7th day along with general behavior pattern by exposing each animal to open area. On 90th day, all the animals of Group-I to Group-IV were kept for overnight fasting. Next day, blood was collected by supraorbital puncture with the help of microcapillary tubes under light ether anesthesia for the estimation of serum (Sr.) biochemical and hematological parameters. The final body weight of each animal was noted and thereafter sacrificed. The abdomen was opened through a middle incision, and records of gross autopsy (examination of the viscera and general animal profile at the time of the sacrifice involving external examination of the animal - different parts such as head, body, and limbs; nature of discharges from natural orifices such as mouth, nose, vagina, anus and penis; internal examination of the buccal cavity with its organs; abdominal cavity and its organs, pelvic cavity with its organs) were maintained followed by dissecting out the important organs.

Additional six animals were kept in TED × 10-treated group (TED × 10 recovery group) for observation after the treatment period, for reversibility or persistence of any toxic effects. The duration of this posttreatment period was fixed for 30 days (a total of 120 days including 90-day treatment period and 30-day recovery period). During this period also, body weight and general behavior pattern were recorded.

Hematological parameters were performed in the laboratory by using an automatic hematological analyzer. Total white blood cells (WBC) count, different WBC count (neutrophils, lymphocytes, eosinophils, monocytes and basophils), hemoglobin, packed cell volume, total red blood cells (RBCs), platelet count, mean corpuscular volume, mean cell hemoglobin (MCH) and MCH concentration were measured from the fresh blood samples of animals.

Sr. biochemical parameters were carried out by using fully automated biochemical random access analyzer in IPGT and RA Pathology Laboratory. Blood sugar, Sr. cholesterol, triglycerides, high-density lipoproteins (HDLs), very low-density lipoproteins (VLDLs), urea, creatinine, uric acid, total bilirubin, direct bilirubin, Sr. glutamic pyruvic transaminase (SGPT), Sr. glutamic oxaloacetic transaminase (SGOT), alkaline phosphate, total proteins, albumin, globulin and calcium[18-28] parameters were examined from the blood samples of the animals.

Ponderal changes were noted in liver, heart, spleen, kidney, uterus, prostate, testis, and seminal vesicle after careful dissection, and all the important organs were transferred to 10% buffered formalin solution for fixation for histopathological evaluation. The slides were viewed under Trinocular Research Carl Zeiss Microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues.[29]

Statistical analysis

The data were expressed as mean ± standard error of the mean for six rats per experimental group. One-way analysis of variance was used to compare the mean values of quantitative variables among the groups followed by Dunnett's multiple "*t*" test and Student's "*t*" test for unpaired data as applicable to determine significant difference between groups at $P < 0.05$.

Results

No any major behavior changes were observed in SPR-treated groups during 90 days of chronic toxicity study in comparison to VC group. No mortality was observed in any of the treated groups. A moderate increase in food intake was observed in SPR-treated groups. Fecal and urine output remained unaffected in all the treated and VC groups.

Normal progressive gain in body weight was observed at all dose levels of SPR at all the time intervals in comparison to their respective initial values during chronic toxicity study, and almost similar trend of weight gain was observed in VC group [Table 1]. Administration of test drug at different dose levels resulted in insignificant changes (in comparison to VC) in the relative weight of different eight organs (liver, heart, spleen, kidney, uterus, testis, seminal vesicle and prostate) [Table 2].

Administration of test drug at TED level resulted in significant increase ($P < 0.05$) in neutrophils and a significant decrease ($P < 0.05$) in lymphocytes in comparison to VC whereas found insignificant in other groups [Table 3]. The result of test drug on Sr. biochemical parameters showed a significant decrease ($P < 0.05$) in uric acid and a significant increase ($P < 0.05$) in alkaline phosphatase in TED × 10 recovery group in comparison to VC, while the change was insignificant in other groups in comparison to the vehicle group [Table 4]. Test drug did not affect liver enzymes SGOT and SGPT at all dose levels in comparison to VC group.

The section of different organs from treated groups for the evaluation of histopathology was compared with sections of VC group under trinocular research microscope at different magnifications. Microscopic examination of liver sections from VC rats exhibited normal cytoarchitecture. Sections of liver [Figure 1], heart [Figure 2], kidney [Figure 3], testis [Figure 4], prostate [Figure 5], uterus [Figure 6] and ovary [Figure 7] from SPR at TED × 10 dose levels in main and recovery studies also exhibited normal cytoarchitecture.

Discussion

Nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes. Body weight indicates the health status of any living being, and an increase in body weight indicates a normal progressive health status of the animals. The drug showed a progressive increase in all treated groups, which nullifies possibility of adverse impact of drug on weight gain (nutritional status, energy balance etc.)

Table 1: Effect of *Sameera Pannaga Rasa* on body weight of albino rats

Group	Body weight (g)				
	Experimental phase				
	Initial	4th week	8th week	12th week	16th week
VC	169.66±10.34	222.33±11.82	261.66±19.49	266.50±15.91	-
SPR TED	194.33±3.93	235.00±16.69	283.66±29.51	310.83±37.37	-
SPR TED × 5	194.40±3.58	250.20±11.22	278.60±18.02	310.20±26.89	-
SPR TED × 10	188.66±3.59	239.50±9.65	259.50±11.90	288.33±20.60	-
SPR TED × 10 R	186.80±2.87	227.40±12.13	259.80±23.90	294.40±37.68	279.40±36.26

Data presented as mean±SEM. SEM: Standard error of mean, VC: Vehicle control, SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose

Table 2: Effect of *Sameera Pannaga Rasa* on relative organ weight of albino rats

	Sameera Pannaga Rasa				
Relative weight	VC	TED	TED × 5	TED × 10	TED × 10 R
Heart (mg/100 g BW)	308.69±8.69	279.35±13.17	261.26±15.83	301.78±17.97	265.29±13.73
Liver (g/100 g BW)	3.22±0.12	3.28±0.09	2.90±0.07	2.90±0.08	2.99±0.24
Spleen (mg/100 g BW)	215.86±17.88	215.43±15.52	193.43±20.09	175.65±13.12	172.42±15.71
Kidney (mg/100 g BW)	622.07±36.17	572.82±46.69	568.76±30.84	558.69±15.02	596.00±12.52
Uterus (mg/100 g BW)	273.91±24.81	250.57±29.87	226.74±21.28	264.36±33.67	290.26±58.28
Testis (mg/100 g BW)	955.28±80.73	652.37±116.51	771.62±31.39	855.10±39.96	731.17±36.87
Seminal vesicle (mg/100 g BW)	415.78±98.84	492.90±68.88	273.53±24.64	293.08±66.53	357.61±78.13
Prostate (mg/100 g BW)	188.05±31.18	241.91±34.12	173.73±27.28	114.03±4.89	157.66±27.53

Data presented as mean±SEM. SEM: Standard error of mean, VC: Vehicle control, TED: Therapeutic dose

Table 3: Effect of *Sameera Pannaga Rasa* on hematological parameters of albino rats

Parameters	VC	TED	TED × 5	TED × 10	TED × 10 R
WBC (10^3/Cu mm)	9383.33±1355.58	9283.33±587.320	9140.00±1057.16	7760.00±653.91	9500.00±945.51
Neutrophil (%)	12.50±1.23	20.00±2.08$^{\$}$	18.40±2.69	17.60±1.20	9.00±1.51
Lymphocytes (%)	83.50±1.76	75.83±2.24*	77.60±2.94	78.00±1.67	87.20±2.41
Eosinophils (%)	2.16±0.30	2.00±0.25	2.40±0.24	2.20±0.20	1.80±0.37
Monocyte (%)	1.83±0.30	2.16±0.30	1.60±0.24	2.20±0.37	2.00±0.63
Hemoglobin (%)	15.06±0.27	14.95±0.29	15.58±0.30	15.82±0.40	0.00±0.00
PCV (%)	46.21±0.94	46.45±0.96	48.18±1.27	47.78±1.55	47.16±2.00
RBC (mil/cumm)	8.46±0.32	8.49±0.24	8.62±0.26	8.60±0.32	8.35±0.40
Platelet count (10^3/ul)	1244.50±63.86	1269.83±36.96	1120.40±16.77	1150.20±54.07	1062.60±82.78
MCV (fl)	54.76±0.96	54.73±0.77	55.90±0.65	55.58±0.51	56.52±0.78
MCH (pg)	17.81±0.38	17.63±0.33	18.08±0.25	18.44±0.36	18.30±0.17
MCHC (g/dL)	32.60±0.14	32.23±0.31	32.36±0.32	33.14±0.41	32.48±0.39

$^{\$}P<0.05$, when compared to vehicle control group (ANNOVA followed by Dunnett's multiple "*t*"-test), *$P<0.05$ when compared with vehicle control group (unpaired "*t*"-test). Data: Mean±SEM. SEM: Standard error of mean, VC: Vehicle control, TED: Therapeutic dose, WBC: White blood cells, PCV: Packed cell volume, RBC: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration

in comparison with the VC group. The drug did not affect the relative weight of heart, liver, spleen, kidney, prostate, seminal vesicle, testis, and uterus significantly at all dose levels in comparison with the VC group in albino rats. Hence, an increase in body weight till the period of drug administration in TED and till recovery period may suggest safety of drug not only on mass balance but also on body functions as a whole which is also supported with insignificant and or nonconsistent changes in functional biomarkers for different organs and systems.

The test drug at all dose levels did not affect RBC-related parameters significantly. It indicates that the test drug did not affect the cellular and noncellular elements of blood to a significant extent in comparison with the VC group, suggesting that drug does not affect hemopoiesis and maturation of pleuripotent cells of the bone marrow. Impairment of hemopoiesis is a feature of arsenic toxicity, and it gets deposited in bones for long duration, but the findings of present study deny any possibility of such adverse effects of SPR.

Table 4: Effect of *Sameera Pannaga Rasa* on serum biochemical parameters of albino rats

Parameters	VC	TED	TED × 5	TED × 10	TED × 10 R
Blood sugar (mg/dL)	89.00±9.21	88.00±3.53	85.60±4.44	88.20±6.35	101.80±3.02
Cholesterol (mg/dL)	52.66±4.06	47.00±4.26	57.60±3.65	56.60±5.75	55.60±3.25
Triglyceride (mg/dL)	139.66±20.77	98.33±13.98	116.00±31.92	132.20±34.05	137.40±37.96
HDL (mg/dL)	41.66±2.15	38.00±3.47	41.60±4.47	48.00±3.84	41.00±3.88
VLDL cholesterol (mg/dL)	28.00±4.27	19.67±2.80	23.00±6.30	26.40±6.82	27.60±7.54
Urea (mg/dL)	40.16±2.65	39.83±2.46	36.00±3.39	42.80±3.91	40.20±3.59
Creatinine (mg/dL)	0.68±0.04	0.68±0.04	0.80±0.05	0.86±0.06	0.78±0.04
Uric acid (mg/dL)	0.93±0.09	0.85±0.08	0.76±0.06	0.76±0.04	0.64±0.07*
Bilirubin (T) (mg/dL)	0.30±0.04	0.23±0.02	0.32±0.04	0.30±0.03	0.24±0.02
Bilirubin (D) (mg/dL)	0.13±0.03	0.10±0.00	0.16±0.04	0.12±0.02	0.12±0.02
SGPT (IU/L)	62.66±3.80	63.00±5.09	54.20±3.70	61.80±6.51	51.80±5.58
SGOT (IU/L)	110.50±4.49	113.00±8.70	113.00±8.39	111.20±5.47	103.00±10.35
Alkaline phosphatase (IU/L)	98.33±15.78	122.16±10.54	99.00±14.74	94.00±8.99	150.20±10.97$
Total protein (g/dL)	7.20±0.14	6.75±0.29	7.22±0.20	7.36±0.26	7.22±0.19
Albumin (g/dL)	3.68±0.25	3.51±0.26	3.56±0.33	3.94±0.13	3.92±0.18
Globulin (g/dL)	3.51±0.22	3.23±0.13	3.66±0.24	3.42±0.18	3.30±0.16
Calcium (mg/dL)	9.95±0.13	9.75±0.19	9.74±0.22	10.10±0.31	10.18±0.24

*$P<0.05$ compared with vehicle control group (Unpaired "*t*"-test), $$P<0.05$ when compared to vehicle control group (ANNOVA followed by Dunnett's multiple "*t*"-test). Data: Mean±SEM. SEM: Standard error of mean, VC: Vehicle control, TED: Therapeutic dose, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase

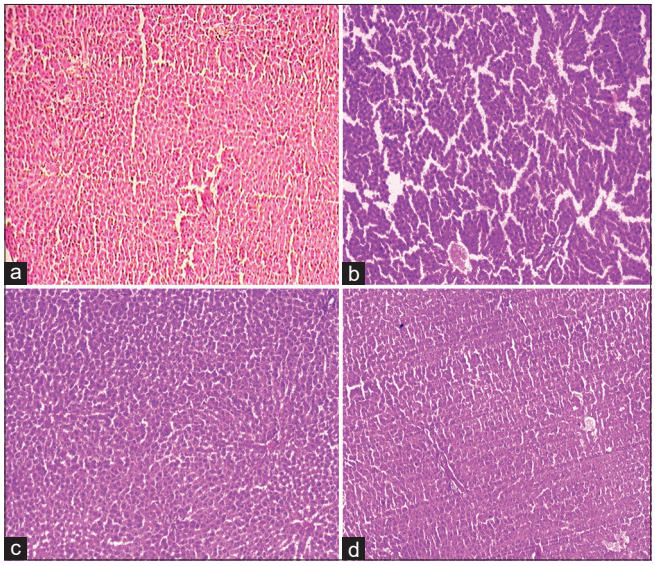

Figure 1: Photomicrographs of representative sections of the liver of albino rats at ×100: (a) Normal cytoarchitecture in vehicle control group, (b) normal cytoarchitecture in SPR TED ×10, (c) normal cytoarchitecture in SPR TED ×10 R, (d) normal cytoarchitecture in SPR TED ×10 R at ×101. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

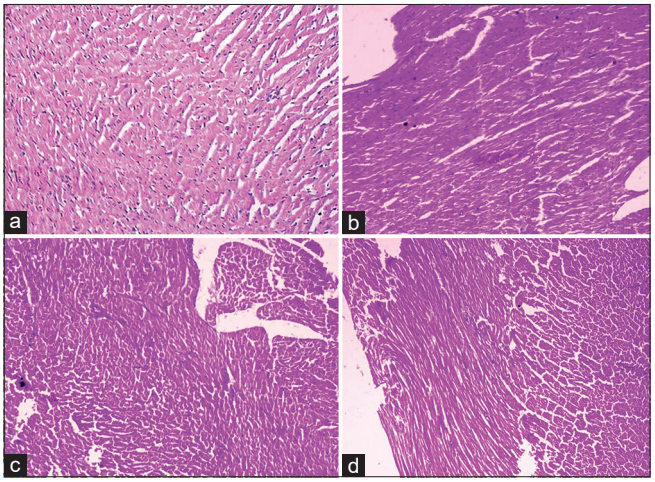

Figure 2: Photomicrographs of representative sections of the heart of albino rats at ×200: (a) Normal cytoarchitecture in vehicle control group, (b) normal cytoarchitecture in SPR TED ×10, (c) normal cytoarchitecture in SPR TED ×10 R, (d) normal cytoarchitecture in SPR TED ×10 R at ×201. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

The elemental or metallic mercury, inorganic mercury, and organic mercury are associated with major biological damage in the body. Altered glucose level is one form of this damage, as exposure to mercury and all forms has been shown to promote DNA-damaging oxidative stress. Organic or inorganic compounds are well known to induce cellular damage in various cell types, such as renal cells, astrocytes, human gingival fibroblast cells, alveolar epithelial cells, and pancreatic islet beta cells.[30] Arsenic is a highly toxic metalloid which causes the nervous system disorders, peripheral vascular disease, endocrine disruption, and cancer; it has also been found to alter certain biological processes that regulate insulin resistance. Thus normal blood sugar levels and histopathology of pancreas, brain nullifies toxicity due to merccury and arsenic.[31]

Lipid metabolism to a great extent depends on the formation and turnover of lipoproteins. Almost all lipids in the plasma are transported in the form of complexes with proteins; these proteins are termed as lipoproteins. They are particles with a core region containing cholesterols esters and triglycerides. Chylomicrons are the largest of the lipoproteins which are formed in the

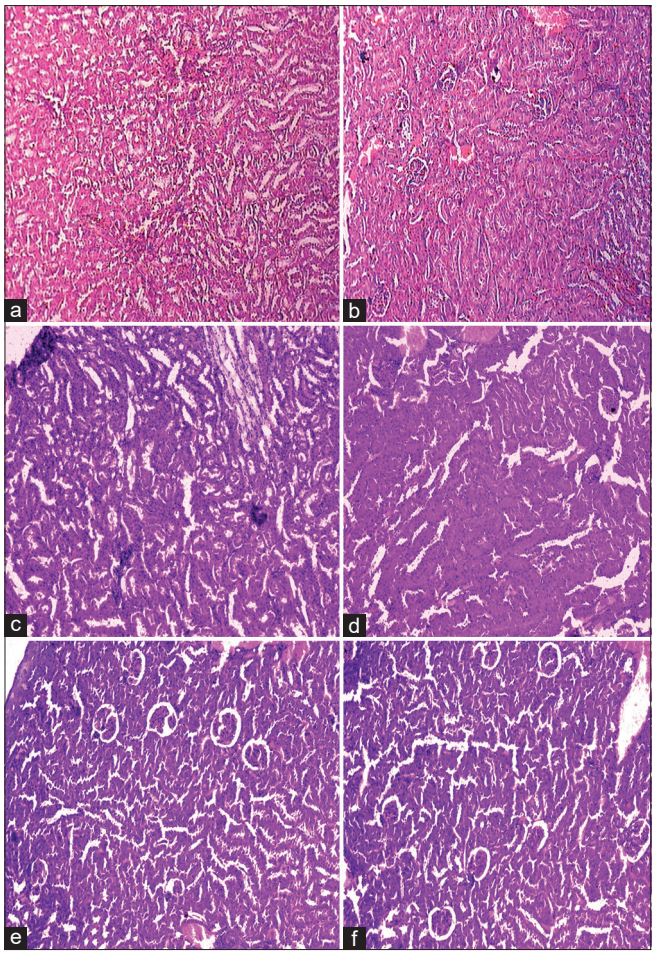

Figure 3: Photomicrographs of representative sections of the kidney of albino rats: (a) Normal cytoarchitecture in vehicle control group at ×100, (b) normal cytoarchitecture in vehicle control group at ×101, (c) normal cytoarchitecture in SPR TED ×10 at ×100, (d) normal cytoarchitecture in SPR TED ×10 at ×101, (e) normal cytoarchitecture in SPR TED ×10 R at ×100, (f) normal cytoarchitecture in SPR TED ×10 R at ×101. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

intestine and carry triglyceride from dietary region. After the triglyceride depletion, the remaining surface lipids and protein components are transferred to HDL moiety and the remaining chylomicron remnants are taken up by the hepatocytes. VLDL is secreted by the liver. They transport like chylomicrons to extrahepatic tissues. Depletion of triglyceride from VLDL leads to the formation of intermediate-density lipoprotein (IDL). Part of IDL is taken up by the hepatocytes and protein is converted to low-density lipoproteins (LDLs). The protein part of HDL comes from the liver and intestine. The lipid portion of HDL comes from the surface layer of chylomicrons and VLDL when they are hydrolyzed. It also takes up cholesterol from the cells. From HDL, cholesterol is transferred to VLDL, IDL, LDL, and chylomicron remnants with the aid of cholesteryl ester transfer protein. Thus, cholesterol ends up finally in the liver.

There were insignificant changes in cholesterol level at all dose levels in comparison to VC group at the end of 3 months as

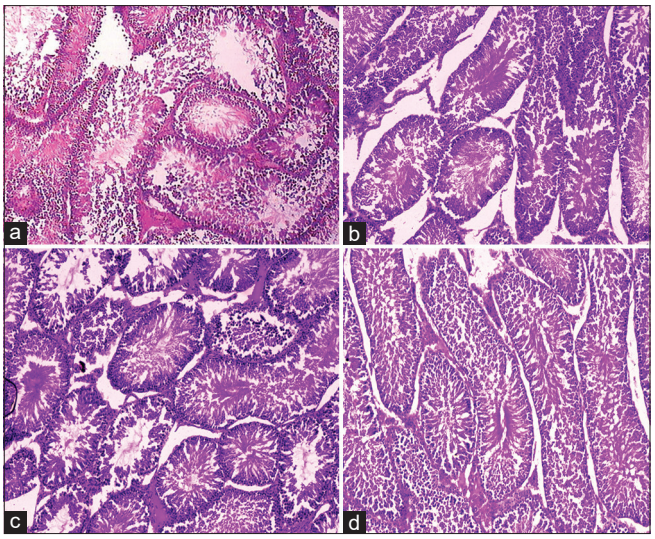

Figure 4: Photomicrographs of representative sections of the testis of albino rats: (a) Normal cytoarchitecture in vehicle control group at ×100, (b) normal cytoarchitecture in SPR TED ×10 at ×200, (c) normal cytoarchitecture in SPR TED ×10 at ×201, (d) normal cytoarchitecture in SPR TED ×10 R at ×200. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

well as in recovery study, suggesting safety of SPR on lipid metabolism as well as on hepatic function.

A nonsignificant increase in albumin level and decrease in globulin level at higher dose suggest the role of test drug on protein metabolism. The liver's ability to synthesize albumin and globulin is reduced if the synthetic function of the liver is hampered. The values of protein content and its constituent are still within the normal range. Although albumin: globulin ratio in all test drug received groups is at lower range, still Sr. albumin is within normal limits without hypoalbuminemia which assures safety of drug on hepatic function.

The transaminases (SGOT and SGPT) are popular enzymes used as biomarkers for the anticipation of possible hepatic toxicity or damage. Elevations in SGPT are usually associated with cell necrosis in many tissues (skeletal or cardiac muscles or hepatic parenchyma). Since it is one of the specific assayable liver enzymes and its elevation is associated with liver damage, it is well known that the liver metabolizes a wide range of both exogenous and endogenous compounds and acts as a pointer of the detoxification process in the organisms. SPR after discontinuation in recovery study again found nonsignificant in SGPT and SGOT level in comparison to VC group.[32]

Sr. alkaline phosphatase is found in most of the tissues. However, the osteoblasts in the bone, bile canaliculi in the liver, epithelial cells in the intestine, proximal tubules of the kidney, placenta, and lactating mammary glands are the richest sources. In the present study, nonsignificant increase was found in TED, but the change is not consistent as there is no increment in 5 × TED group whereas noticed decreased in 10 × TED group in the main study while a significant increase was noted after discontinuation of test drug in recovery study.[33] As in higher

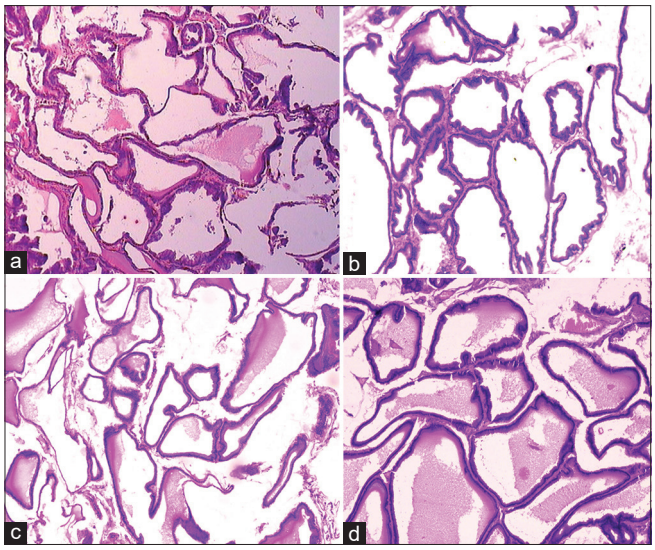

Figure 5: Photomicrographs of representative sections of prostate of albino rats at ×100: (a) Normal cytoarchitecture in vehicle control group, (b) normal cytoarchitecture in SPR TED ×10, (c) normal cytoarchitecture in SPR TED ×10 at ×101, (d) normal cytoarchitecture in SPR TED ×10 R at ×100. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

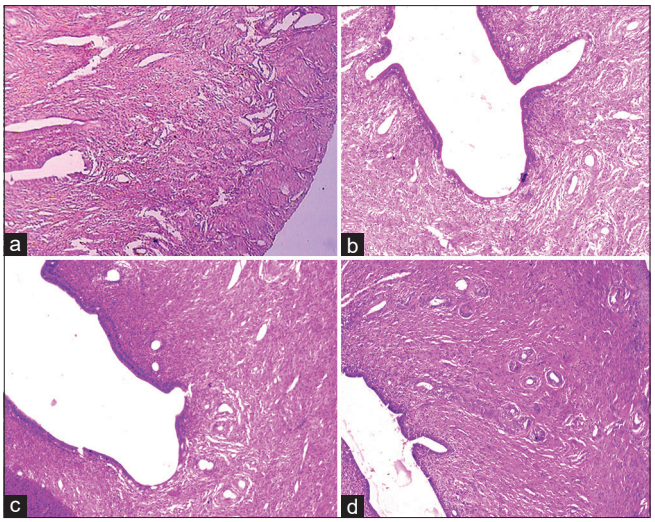

Figure 6: Photomicrographs of representative sections of the uterus of albino rats at ×100: (a) Normal cytoarchitecture in vehicle control group, (b) normal cytoarchitecture in SPR TED ×10, (c) normal cytoarchitecture in SPR TED ×10 at ×101, (d) normal cytoarchitecture in SPR TED ×10 R at ×100. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

dose (10 × TED), Sr. alkaline phosphate noticed decreased, significant increase in recovery study has less significance and nullifies chances of significant alteration of physiology of above-cited organs y SPR at all dose levels. Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen, and bone marrow. An increase in the tissue or Sr. bilirubin concentration results in jaundice and it occurs in toxic or infectious disease of the liver, e.g. hepatitis or bile obstruction. Bilirubin measurement is also a useful index of determining the excretory function of the liver and assessment of hemolytic anemia; further, it indicates the hemolytic action. Bilirubin production increases in inefficient erythropoiesis, hemolysis, re-absorption of hematoma, and rarely muscle injury. SPR at all dose levels produced no effect in bilirubin level.[34]

Non-significant alteration of biochemical markers of liver (enzymes; SGPT, SGOT, Sr. bilirubin, Sr. proteins, albumin/globulin ratio, Sr. alkaline phosphate, lipid profile, blood glucose, etc.) suggests its safety against above-cited organs specifically liver, above-cited biological processes, and other biological processes such as protein, lipid, sugar metabolism, metabolism of bile salts and bile pigments which are likely to get affected by arsenicals and mercurials and suggests safety of drug against damage to the pancreas, owing to insignificant alterations in blood sugars. Liver is an organ responsible for several biological processes and especially drug and food metabolism. Non-alteration of its biomarkers is assurance of drug safety of these biological processes.

Urea is the main product of protein metabolism in the body. The kidney plays an important role in balancing the urea level. Increase in Sr. uric acid is seen idiopathically in renal failure along with many other conditions. Nonsignificant increase in urea in SPR-treated group at all dose levels may indicate mild impairment in the functioning of the kidney. However, the significant decrease was observed in uric acid, in SPR-treated TED × 10 group after discontinuation of the drug. There was insignificant alteration in urea content in comparison to VC group in albino rats. Decreased Sr. uric acid level may not be of clinical significance. In the main study, the test drugs do not affect the level of urea and uric acid at a significant level in comparison to VC group which suggest that test drug did not affected the kidney functions in rats.[35]

Creatinine is synthesized from three amino acids namely arginine, glycine, and methionine in the liver and then transported by blood to muscle and brain. Its blood level depends on its production and excretion. It also depends on the production of creatine phosphate in muscle mass. SPR at all dose level produced no significant effect on serum creatinine level, which suggests that test drug does not have any adverse effect on metabolism of creatinine. Kidney is an important organ of excretory system and is a vital organ which mainly excretes almost all drugs; hence, nephrotoxicity is common presentation of drug toxicity and nephrotoxicity is a presentation of chronic toxicity of arsenic and mercury. Insignificant alteration in biomarkers of kidney function (Sr. creatinine, blood urea, Sr. uric acid) assures safety of SPR in view of nephrotoxicity.

SPR at all dose levels produced no effect in Sr. calcium level in main and recovery study nullifying possibility of alteration of calcium turnover in the body.

Sections of different organs from different groups were examined under trinocular research microscope at different magnifications to note down the changes in the cytoarchitecture

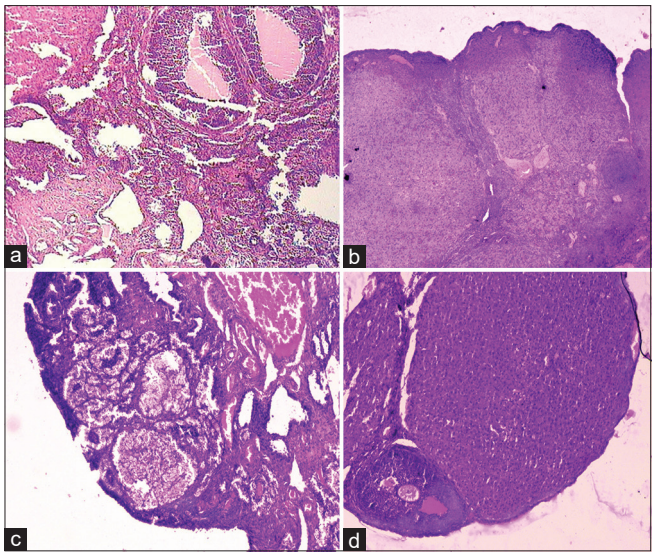

Figure 7: Photomicrographs of representative sections of ovary of albino rats at ×100: (a) Normal cytoarchitecture in vehicle control group, (b) normal cytoarchitecture in SPR TED ×10, (c) normal cytoarchitecture in SPR TED ×10 R, (d) normal cytoarchitecture in SPR TED ×10 R at ×101. SPR: *Sameera Pannaga Rasa*, TED: Therapeutic dose, R: Recovery

of the organs if any. The section of different organs from the treated group was compared with sections of normal control group.

The histopathological studies of the organs showed that SPR along with adjuvant honey produced no adverse changes in cytoarchitecture of liver, heart, spleen, kidney, uterus, prostate, testis and seminal vesicle of albino rats in comparison to VC group even at ten times higher dose level. Test drug at a higher dose level and recovery study showed normal changes in comparison to VC group in albino rats during chronic toxicity study.

Conclusion

SPR administered with honey is found to have no toxic effect (on behavioral, ponderal parameters, functional biomarkers, and cytoarchitecture of studied eight organs) in albino rats during the repeated dose, oral, choric toxicity study of 90 days even at 10 times TED (112.25 mg/kg) and even during recovery period of 1 month. Hence, SPR is considered a safe medicine at TED 11.25 mg/kg in albino rats, i.e., adult human dose of 125 mg/day for oral administration till 90 days.

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Conflicts of interest

There are no conflicts of interest.

References

1. Borkar D, Raschandanshu. Ver. 1258-60. New Delhi: CCRAS. 2011, p. 280.
2. Gune G. Ayurveda Aushadhi Gun Dharma Shastra. 1st ed. Ahmadnagar: Vaidyak Grantha Bhandar; 2005. p. 514.
3. Anonyms. Ayurvedic Formulary of India. Part 1., 2nd ed. New Delhi: Ministry of Health and Family Welfare, Government of India, Department of AYUSH; 2003. p. 212.
4. Deshpande S, Gandhi N. Drugs and cosmetic act, 1940 ad Rules 1945 The Medical devices rules 2017. 9th ed. Mumbai: Susmit publishers. 2018, p. 462.
5. Krishnanand, Rasatantrasara, and Siddha Prayog Sangraha. Kupipakwa Prakarana. 8th ed. Ajmer: Krishna Gopal Ayurved Bhawan Publishers; 2010. p. 138.
6. Acharya YT, editor. Charaka Samhita of Agnivesha, Chikitsa Sthana. Ch. 17, Ver. 11. Reprint edition. Varanasi: Chaukhambha Publications, 2018. p. 533.
7. Rao M, Kar A, Bhattacharya P, Hazra J. Clinical evaluation of Efficacy of Pippali Vardhamana Ksheerapaka, Sameerpannaga Rasa in the management of Amavata (Rheumatoid Arthritis). JRAS, 2005;26:1-2, p. 1-15.
8. Pierre Boutrand, Jean, De Jong WH, Geertsma RE, editor. Biocompatibility and Performance of Medical Devices. Cambridge, UK; Woodhead publishing, Elsevier publications; 2012. p. 120-58.
9. Shastri Kashinath, editor. Rasatarangini by Sadananda Sharma, 5th Taranga. Ver. 27-28. Reprint edition. New Delhi: Motilal Banarasidas; 2012. p. 79-80.
10. Kulkarni Dattatraya Ananta, editor. Rasaratnasamucchaya by Vagbhatacharya. Ch. 3., Ver. 20-22. New Delhi: Meherchanda Lachamandasa Publications; 2009. p. 45-6.
11. Prof. Dattatraya Ananta Kulkarni Rasaratnasamucchaya of Vagbhatacharya. Ch. 3, Ver. 26-27. New Delhi: Meherchanda Achamandasa Publications; 2009. p. 61-2.
12. Shastri Kashinath, editor. Rasatarangini by Sadananda Sharma, 11th Taranga. Ver. 137-28. Reprint edition. New Delhi: Motilal Banarasidas; 2012. p. 237.
13. Shastri Kashinath, editor. Rasatarangini by Sadananda Sharma, 11th Taranga. Ver. 137-28. Reprint edition. New Delhi: Motilal Banarasidas; 2012. p. 262.
14. Sharma M, Bedarkar P, Patgiri BJ. Standard operating procedure (SOPs) of *Sameera Pannaga Rasa* prepared through vertical electric muffle furnace. WJPR 2019;8:884-94.
15. Paget GE, Barnes JM, editors. Evaluation of drug activities. Pharmacometrics. Vol. 1. New York: Academic Press; 1964. p. 161.
16. Anonymous. OECD 408, Repeated Dose 90-day Oral Toxicity Study in Rodents, OECD Guideline for The testing of chemicals, 1998. Available from: https://www.oecd.org/chemicalsafety/testing/Revision-OECD-TG408-repeated-dose-90-day-oral-toxicity-study-in-rodents.pdf (last accessed on 24.05.2021).
17. Anonymous. General Guidelines for Safety/Toxicity Evaluation of Ayurvedic Formulations. New Delhi: Ministry of AYUSH, Govt. of India; 2018. Available from: https://www.ayush.gov.in/docs/guideline_safety_toxicity.pdf. [Last accessed on 2020 Nov 19].
18. Tiffany TD, Janseen JM, Burtis CA, Overton JB, Scott TD. Enzymatic kinetic rate and end point analysis of substrate by use of a GeMSAEC fast analyzer. Clin Chem 1972;18:829-40.
19. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest 1965;17:381-7.
20. Tietz NW. Text Book of Clinical Chemistry. London: W.B. Saunders; 1986. p. 579.
21. Doumas BT, Arends RL, Pinto PC. Standard Methods of Clinical Chemistry. Vol. 7. Chicago: Academic Press; 1972. p. 175-89.
22. Wilkinson JH, Boutwell JH, Winsten S. Evaluation of a new system for kinetic measurement of serum alkaline phosphatase. Clin Chem 1969;15:487-95.
23. Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders; 1995. p. 76.
24. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia, PA: Moss D.W., Henderson A.R; 1999. p. 652.
25. Pearlman PC, Lee RT. Detection and measurement of total bilirubin in serum with use of surfactants as solubilising agents. Clin Chemi 1974;20:447.
26. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia, PA: WB Saunders; 1999. p. 1136.

27. Tietz NW. Text Book of Clinical Chemistry. Philadelphia: WB Saunders; 1986. p. 579.
28. Kabasakalian P, Kalliney S, Wescott A. Determination of uric acid in serum, with use of uricase and tribromophenol-aminoantipyrinechromogen. Clin Chem 1973;19:522.
29. Raghuramulu N, Nair KM, Kalyanasundaram S. A Manual of Laboratory Techniques. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research; 1983.
30. Available from: http://naturalnews.com/044852_heavy_metals_diabetes_trace_ellements.html#ixzz4GLJYlvy. [Last accessed on 2019 Aug 28].
31. Available from: https://naturalnews.com/044852_heavy_metals_diabetes_trace_elements.html#ixzz4GLK2TRs5. [Last accessed on 2019 Aug 28].
32. Fenkel JM, Navarro VJ. Herbal and dietary supplement-induced liver injury. Gastroenterol Hepatol 2011;7:695-6.
33. Das AK. Medical Physiology. Vol. 2. Kolkata: Books and Allied Ltd; 2001. p. 119.
34. Eden DO, Usoh IF. Biochemical changes in Wistar Rats on oral doses of Mistleto (*Loranthus micranthus*).Am J Pharmacol Toxicol 2009;4:94-7.
35. Friedman RB, Young DS Effects of Disease on Clinical Laboratory Tests, American Association of Clinical Chemistry. 3rd ed. Washington, DC: AACC Press; 1980. p. 13-24.