Ninety days repeated dose oral toxicity study of *Makaradhwaja* in Wistar rats

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Abstract

Context: *Makaradhwaja* is a *Kupipakwa Rasayana*. Since it contains two heavy metals, namely mercury and gold, it is essential to evaluate its safety. Hence, the present study was undertaken with an objective to evaluate toxicity and target organ of toxicity of *Makaradhwaja* if so. **Aims:** The objective was to evaluate toxicological profile, the target organ of toxicity and to find no observed effect level (NOEL) or no observed adverse effect level (NOAEL) in rats after oral administration for ninety consecutive days. **Materials and Methods:** *Makaradhawaja* preparation was administered to male and female Wistar rats for ninety consecutive days at 2.7, 13.5, and 27 mg/kg body weight. All relevant biochemical and hematological changes were observed. At termination, all the rats were sacrificed and necropsy was performed. Histopathological evaluation was also performed. **Statistical Analysis Used:** Dunnett's test followed by analysis of variance. **Results:** There was a significant increase in high-dose group kidney weight of both sexes which could not be correlated with histopathology findings and serum biochemistry. Therefore, the change was not considered as an adverse effect. **Conclusions:** The dose level 27 mg/kg of *Makaradhwaja* was found as NOAEL and dose level 13.5 mg/kg of *Makaradhwaja* was found as NOEL.

Keywords: Gold, *Makardhwaja*, mercury, rats, toxicity

Introduction

Makaradhwaja is a classical Ayurvedic preparation categorized as *Kupipakwa Rasayana* (rejuvenating mercurial formulation which is prepared in glass bottle by sublimation through sand bath with specific temperature pattern). The oldest reference of its use is found in classical text of *Rasendra Chintamani* by *Acharya Dhundhuknath* in the 16th century.[1] According to Chopra,[2] its first reference can be attributed to *Vaidya Bhva Mishra* of 16th century. *Kupipakwa Rasayana* is basically powdered minerals and metals heated gradually in glass flask. They have often characteristic red (or yellow color) and their potency persists indefinitely if stored in well-stoppered bottles.[3] Gold and mercury are the heavy metal contents of the *Makaradhwaja* along with sulfur.[4] *Makaradhwaja* is aphrodisiac and nutrient to body and mind with adapto-immuno-neuro-endocrino-modulator properties. It is indicated for dyspepsia, weakness of heart, senility and fever.[4]

Users of Ayurvedic medicine may be at risk for heavy metal toxicity.[5] Repeated exposure to heavy metal containing products may lead to cumulative toxicity. Regulatory guidelines also require the product to be tested for its toxicity in rodents carrying single and repeated dose studies so as to establish the no observed effect level (NOEL) and no observed adverse effect level (NOAEL).[6] The present study was a part of multicentric toxicity studies of the Central Council for Research in Ayurvedic Sciences, hence drug was provided in a coded manner.

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Materials and Methods

Test drug preparation and analysis

Makaradhwaja was provided by the Central Council for Research in Ayurvedic Sciences, New Delhi, in a coded manner that was decoded along with chemical analysis report after completion of the study. The drug was prepared as per the

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method described in *Rasa Tarangini*[7] a classical Ayurvedic text.

Details of the ingredients are given in Table 1.

All the ingredients were collected and the final drug was as given below.

Purified gold was taken into mortar and purified mercury was added to it and triturated. Trituration was continued till amalgam of *Swarna* (gold) and *Parada* (mercury) was formed. The amalgam of *Swarna* and *Parada* and powder of *Shodhit Gandhaka* (purified sulphur) in specified quantity was taken and grinded to obtain homogenous, soft, fine (non lustrous) powder. The fresh juice of flower of *Rakta Karpasa* (Gossypium arboreum Linn) was prepared and added to the *Swarna Kajjali* in adequate quantity and levigated. After completing the process, whole mixture was dried. The fresh juice of root bark of *Ankol* (*Alangium salvifolium* Linn.) was prepared and added to the *Swarna Kajjali* in adequate quantity and levigated. After completing the process, the whole mixture was dried. Pulp collected from fresh leaves of *Kumari* (*Aloe vera* Linn) was processed to obtain fresh juice. The fresh juice was added to the *Swarna Kajjali* in adequate quantity and levigated. After completing the process, whole mixture was dried. The dried *Swarna Kajjali* was taken in a glass bottle and placed in *Valuka Yantra* (sand bath). Then, the *Valuka Yantra* was subjected to heat adopting *Kupipakva* method. The temperature was increased gradually in a phased manner. At the end, *Valuka Yantra* was allowed to self cool and bottles were taken out. The bottles were observed for deposition of *Makaradhwaja* at the neck (i.e., *Kanthastha Makaradhwaja*). A thread soaked in kerosene was tied around the bottle just below the level of deposited *Makaradhwaja* and burnt to break the bottle. After this, *Kanthastha Makaradhwaja* was collected in mortar and was grinded to convert it in to fine powder, which was reddish (*Sindoor Varneeya*) in color. The residual of gold obtained from the bottom of the bottles was collected separately.

Physicochemical analysis, namely morphological description, estimation of moisture content, qualitative elemental testing, and Ayurvedic parameters, were carried out by following standard methods as per the Ayurvedic Pharmacopoeia of India Guidelines.

The quantitative elemental composition was performed on JY 2000 sequential inductively coupled plasma-atomic emission spectrometry (ICP-AES) spectrometer (Horiba Jobin Yvon, France). The operating parameters of ICP-AES were – RF power: 1000 w at 40.68 MHz, plasma gas flow rate: 12 L/min, nebulizer gas flow rate: 0.1 L/min, Sample uptake rate: 1.2 L/min, slit with: 20 micron/20 micron (entrance/exit), and monochromator: 0.64 m focal length, 2400 groves/mm, Czerny turner mounting.

Table 1: Ingredients used for preparation of *Makaradhwaja*

Ingredients	Latin name	Part used	Quantity
Shodhit Swarna	Gold	-	1 part
Shodhit Parada	Mercury	-	8 part
Shodhit Gandhaka	Sulphur	-	16 part
Juice of *Raktakarpas* flower	*Gossypium arboreum* Linn	Flower	QS
Juice of *Ankol* root bark	*Alangium salvifolium* Linn	Root bark	QS
Juice of *Kumari*	*Aloe vera* Linn	Leaf	QS

QS: Quantum sates

Study protocol

The study protocol was provided by the sponsor Central Council for Research in Ayurvedic Sciences, New Delhi, which was designed by broadly following principles of Schedule Y of Drugs and Cosmetic Act, 1940.

Housing and environment

A total of 80 Wistar rats (40 male and 40 female) with body weight ranging from 150 g to 200 g were obtained from animal house. Ethical clearance was taken from the Institutional Animal Ethical Committee (IAEC) with letter number 6-17/2003-CRI/Tech/777 dated 02.07.2009. All the rats were maintained as per the guidelines of the committee for purpose of control and supervision of experiments on animals for laboratory animal facility. Rats were acclimatized for 7 days. Temperature and relative humidity were maintained at 25 ± 1°C and 40%–70% respectively and illumination was controlled to give approximately a sequence of 12 h light and 12 h dark. Rats were individually housed in polycarbonate cages (43 cm × 28 cm × 21 cm) with lids and rice husk bedding. Pelleted rodent diet obtained from National Institute of Nutrition, Hyderabad, was provided along with deionized water using plastic bottles with stainless steel nozzle *ad libitum*. Females selected were nulliparous and nonpregnant.

Experimental study design

The animals were divided into four groups of 10/sex/group. Ninety days repeated dose oral toxicity study of *Makaradhwaja* was conducted by daily single administration of the drug at 27 mg/kg (high dose [HD]), 13.5 mg/kg (mid-dose) and 2.7 (low dose) mg/kg body weight along with vehicle control. The test drug was administered as suspension in vehicle, that is, honey mixed with water by gavage and control group received the vehicle. To make the drug and vehicle suspension easy to gavage, it was diluted with water in 2:3 ratio. The suspension was administered @10 ml/kg body weight. The dose calculation was as follows:

Therapeutic dose for human (70 kg body weight approximately): 30 mg/day. Dose conversion factor from human to rats as per the Paget and Barnes[8] is 0.018.

Hence, the dose for rats (200 g body weight approximately) will be 30 × 0.018 = 0.54 mg. Hence, therapeutic equivalent dose for rats per kg/body weight will be 2.7 mg which was considered as low dose. The dose that was 5 times more than therapeutic, that is 2.7 × 5 = 13.5 was considered as mid-dose, and 10 times therapeutic dose, that is, 2.7 × 10 = 27 was considered as highest dose for the study.

All animals were observed for morbidity and mortality twice daily. General clinical observations were made twice a day at

the same time throughout the study. The animals were observed for changes in skin, fur, eyes, mucous membrane, occurrence of secretions and excretions. For neurological examination, the animals were taken outside the cage in a standard arena and their behavior was recorded. Body weights and feed consumption of each animal were recorded at the start of the study and thereafter at weekly intervals. At the termination of the study, that is, on 90th day, serum glucose, total protein, SGOT, SGPT and creatinine along with hematological parameters which were white blood cell and red blood cell count, hemoglobin, hematocrit (%), platelet count, mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration were measured for all animals. Animals were sacrificed on the 91st day using CO_2 euthanasia and were subjected to a detailed postmortem examination and histopathological analysis. The adrenals, heart, kidney, spleen, testis, epididymis, ovaries, uterus, liver, brain and thymus were weighed and collected along with jejunum, duodenum, colon, cecum, ileum, stomach, lungs, pancreas, esophagus and trachea in 10% neutral-buffered formalin. All collected organs from control group and HD group were processed as per the Registry of Industrial Toxicology Animal data guidelines[9] and subjected to histopathological evaluation.

Test drug physicochemical analysis

Makaradhwaja prepared was in the form of reddish powder and had no characteristic odor. Assay of element showed 82% of mercury and 13% of sulphur. Moisture content was 0.08% which was found when loss on drying was determined at 105°C.

Results

No abnormality in clinical signs was detected across all the groups throughout the study. No mortality was found in any of the study groups. No abnormality was detected across the groups during neurological examination. No treatment-related as well as dose-dependent effect on body weights, feed consumption, water consumption, fecal consistency and biochemical parameters was observed as compared to the control group. MCV of HD group male was significantly increased but was within biological limits. However, significant increase in the weights of kidneys from the male and female animals of HD group was recorded. There was no toxicity induced or any other lesion observed during the histopathology evaluation which could be correlated with the increase in weight of the kidneys. Further, there was no significant change in serum biochemistry parameters of HD group females which could be attributed to increased weight of kidneys. The results are described in Tables 2-9. No treatment-related and dose-dependent adverse changes were observed on detailed histopathological evaluation [Figures 1-6]. Photomicrographs of the selected organs/tissues from vehicle control group and high-dose group are provided in Figures 1-6.

Statistical analysis

Study observations such as body weight gain, feed consumption, blood biochemistry and hematology parameters and organ weights were recorded and analyzed statistically. One way analysis of variance and Dunnett's test were applied to compare the dose groups over the control arm.[10]

Discussion

The test drug *Makaradhvaja* was prepared in compliance with Ayurvedic literature and contained heavy metal mercury (82%) and sulphur (13%). However, no treatment related adverse effect was observed up to 10 times therapeutic dose levels, that is, 27 mg/kg body weight in animal experiment. G. Kumar *et al*.[11] studied 28 days repeated dose toxicity of mercury in Ayurvedic formulation of *Aarogyavardhini Vati* in rats at dose of 50, 250, and 500 mg/kg of body weight with mercury content dose equivalent to 1 mg/kg to $HgCl_2$ in HD group and reported that there was no adverse effect on cerebellum,

Table 2: Body weight (g) of male rats

Group	Mean±SD			
	Control	Hd (27.0 Mg/kg)	5Td (13.5 Mg/kg)	Td (2.7 Mg/kg)
Initial body weight	127.24±32.00 (10)	127.20±28.60 (10)	127.10±32.39 (10)	127.46±39.09 (10)
Week 1	153.92±37.23 (10)	155.40±26.23 (10)	165.46±37.46 (10)	160.94±44.41 (10)
Week 2	186.28±29.92 (10)	179.46±26.26 (10)	191.52±36.00 (10)	179.04±48.44 (10)
Week 3	210.18±26.11 (10)	198.80±26.85 (10)	215.14±37.89 (10)	199.76±48.85 (10)
Week 4	231.60±23.84 (10)	220.96±27.40 (10)	233.92±34.35 (10)	217.06±50.20 (10)
Week 5	244.82±24.55 (10)	228.95±30.43 (10)	253.61±36.30 (10)	235.70±56.97 (10)
Week 6	257.42±26.58 (10)	242.68±30.67 (10)	267.92±34.72 (10)	246.62±57.65 (10)
Week 7	269.78±24.70 (10)	256.44±31.59 (10)	279.46±32.85 (10)	258.58±50.84 (10)
Week 8	280.50±27.23 (10)	266.86±33.95 (10)	291.30±32.56 (10)	266.51±51.59 (10)
Week 9	289.20±28.72 (10)	272.88±34.60 (10)	301.10±31.93 (10)	274.72±52.63 (10)
Week 10	296.72±29.55 (10)	274.04±36.91 (10)	308.68±34.20 (10)	276.60±55.87 (10)
Week 11	297.36±28.88 (10)	276.88±40.50 (10)	314.04±33.40 (10)	282.58±51.23 (10)
Week 12	307.62±26.32 (10)	284.20±40.25 (10)	320.94±33.31 (10)	282.12±51.46 (10)
Week 13	311.99±29.12 (10)	289.86±38.26 (10)	325.06±33.27 (10)	279.60±50.10 (10)

Figures in parenthesis indicate number of animal. SD: Standard deviation

Table 3: Body weight (g) of female rats

Group	Mean±SD			
	Control	HD (27.0 mg/kg)	5TD (13.5 mg/kg)	TD (2.7 mg/kg)
Initial body weight	103.34±14.39 (10)	103.36±27.85 (10)	103.08±8.57 (10)	103.34±16.07 (10)
Week 1	111.66±22.36 (10)	118.48±25.61 (10)	113.28±21.03 (10)	129.68±12.20 (10)
Week 2	118.92±24.74 (10)	129.50±21.58 (10)	124.64±17.95 (10)	137.70±11.57 (10)
Week 3	130.28±19.19 (10)	137.42±19.73 (10)	133.26±16.27 (10)	145.88±12.66 (10)
Week 4	134.26±17.74 (10)	144.32±18.23 (10)	140.00±16.85 (10)	151.36±12.34 (10)
Week 5	136.48±19.12 (10)	143.50±19.33 (10)	145.18±15.79 (10)	154.74±11.74 (10)
Week 6	143.30±19.23 (10)	142.42±22.43 (10)	147.68±16.80 (10)	158.30±11.13 (10)
Week 7	149.91±16.66 (10)	144.46±22.33 (10)	153.10±16.90 (10)	162.86±10.84 (10)
Week 8	151.62±16.02 (10)	148.78±26.78 (10)	156.24±18.07 (10)	165.86±10.53 (10)
Week 9	154.24±14.81 (9)	148.16±27.76 (10)	158.46±17.43 (9)	169.08±10.27 (10)
Week 10	154.98±14.92 (9)	150.56±29.50 (10)	160.98±18.62 (9)	167.96±11.26 (10)
Week 11	159.44±14.21 (9)	159.36±20.26 (9)	161.62±17.77 (9)	169.60±11.49 (10)
Week 12	161.93±12.91 (9)	161.44±18.11 (9)	161.76±19.20 (9)	171.02±12.14 (10)
Week 13	162.82±14.24 (9)	162.69±18.55 (9)	163.81±19.59 (9)	170.40±10.73 (10)

Figures in parenthesis indicate number of animals. SD: Standard deviation, HD: High dose, TD: Therapeutic dose

Table 4: Relative organ weight of male rats

	Adrenals	Liver	Brain	Thymus	Heart	Kidneys	Testes	Spleen	Epididymis
Control									
n	10	10	10	10	10	10	10	10	10
Mean±SD	0.03±0.01	2.98±0.31	0.58±0.04	0.07±0.02	0.37±0.03	0.78±0.05	1.01±0.10	0.37±0.05	0.41±0.03
HD (27.0 mg/kg)									
n	10	10	10	10	10	10	10	10	10
Mean±SD	0.04±0.02	3.09±0.30	0.65±0.09	0.09±0.03	0.37±0.03	0.91*±0.06	1.02±0.15	0.34±0.05	0.42±0.05
5TD (13.5 mg/kg)									
n	10	10	10	10	10	10	10	10	10
Mean±SD	0.03±0.01	3.13±0.17	0.60±0.06	0.07±0.01	0.36±0.04	0.87±0.06	0.99±0.15	0.33±0.04	0.42±0.08
TD (2.7 mg/kg)									
n	9	9	9	9	9	9	9	9	9
Mean±SD	0.03±0.02	3.07±0.26	0.71±0.13	0.09±0.03	0.38±0.05	0.87±0.08	1.11±0.12	0.36±0.02	0.41±0.04

*Significant at 5% level. Figures in parenthesis indicate number of animals. SD: Standard deviation, SD: Standard deviation, HD: High dose, TD: Therapeutic dose

Table 5: Relative organ weight of female rats

Group	Adrenals	Liver	Brain	Thymus	Heart	Kidneys	Ovaries	Spleen	Uterus (with cervix)
Control									
n	9	9	9	9	9	9	9	9	9
Mean±SD	0.06±0.04	3.05±0.33	1.05±0.09	0.12±0.06	0.43±0.09	0.84±0.12	0.13±0.15	0.36±0.08	0.31±0.08
HD (27.0 mg/kg)									
n	9	9	9	9	9	9	9	9	9
Mean±SD	0.05±0.02	3.15±0.22	1.04±0.10	0.12±0.04	0.44±0.05	0.96±0.07*	0.09±0.03	0.45±0.06	0.33±0.09
5TD (13.5 mg/kg)									
n	9	9	9	9	9	9	9	9	9
Mean±SD	0.09±0.12	3.16±0.20	1.07±0.11	0.15±0.05	0.41±0.07	0.93±0.09	0.19±0.26	0.43±0.05	0.34±0.08
TD (2.7 mg/kg)									
n	10	10	10	10	10	10	10	10	10
Mean±SD	0.05±0.03	3.19±0.42	1.01±0.06	0.12±0.04	0.41±0.04	0.89±0.07	0.08±0.03	0.44±0.09	0.29±0.06

Figures in parenthesis indicate number of animals. *Significant at 5% level when compared with control group. SD: Standard deviation, SD: Standard deviation, HD: High dose, TD: Therapeutic dose

liver and kidneys and the drug was safe up to 500 mg/kg body weight. Whereas $HgCl_2$ at 1 mg/kg body weight alone showed pyknosis in the brain neurons and congestion of blood vessels was reported in the kidney. G. Kumar *et al*.[11] also attributed the

Table 6: Hematological analysis of male rats at 90th day

Group	WBC (10^3/mm^3)	RBC (10^6/mm^3)	Hb (g/dl)	HCT (%)	PLT (10^3/mm^3)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)
Control								
n	10	10	10	10	10	10	10	10
Mean±SD	5.34±1.83	9.96±2.09	15.78±3.27	49.9±11.41	597.4±136.36	49.7±1.34	15.9±0.91	31.9±1.94
HD (27.0 mg/kg)								
n	10	10	10	10	10	10	10	10
Mean±SD	5.64±2.47	9.49±3.42	16.28±4.24	50.95±15.22	572.7±180.10	51.60±1.17*	16.72±1.08	32.38±2.03
5TD (13.5 mg/kg)								
n	10	10	10	10	10	10	10	10
Mean±SD	5.47±3.25	9.75±3.50	16.31±3.91	51.43±14.80	653.6±156.39	50±1.16	16.09±0.83	32.1±1.67
TD (2.7 mg/kg)								
n	10	10	10	10	10	10	10	10
Mean±SD	5.78±5.32	10.75±1.95	17.24±3.09	54.4±10.55	696.4±190.11	50.7±1.49	16.07±0.84	31.83±1.64

Table 7: Hematological analysis of female rats at 90th day

Group	WBC (10^3/mm^3)	RBC (10^6/mm^3)	Hb (g/dl)	HCT (%)	PLT (10^3/mm^3)	MCV (μm^3)	MCH (pg)	MCHC (g/dl)
Control								
n	9	9	9	9	9	9	9	9
Mean±SD	3.76±2.14	9.82±1.34	16.85±2.39	52.36±7.66	511.13±115.32	53.38±1.92	17.18±0.92	33.08±1.65
HD (27.0 mg/kg)								
n	9	9	9	9	9	9	9	9
Mean±SD	3.5±1.81	8.71±1.56	15.32±2.52	46.3±8.84	509.56±101.78	53.22±1.39	17.68±0.67	33.29±1.24
5TD (13.5 mg/kg)								
n	9	9	9	9	9	9	9	9
Mean±SD	2.43±1.26	8.73±1.58	14.94±2.55	46.22±9.00	522.33±193.94	52.89±1.54	17.12±0.48	32.46±1.25
TD (2.7 mg/kg)								
n	10	10	10	10	10	10	10	10
Mean±SD	3.55±1.79	8.642±1.77	14.9±2.87	45.33±9.84	561.1±195.94	52.3±1.16	17.29±.51	33.04±1.15

Figures in parenthesis indicate number of animals. SD: Standard deviation, WBC: White blood cells, RBC: Red blood cells, Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, HD: High dose, PLT: Platelet, TD: Therapeutic dose

Table 8: Serum biochemical analysis of male rats at 90th day

Group	Glucose (mg/dl)	Total protein (g/dl)	SGOT (U/L)	SGPT (U/L)	Serum creatinine (mg/dl)
Control					
n	10	10	10	10	10
Mean±SD	70.1±21.59	7.18±1.28	218±89.91	62.3±8.18	0.44±0.14
HD (27.0 mg/kg)					
n	10	10	10	10	10
Mean±SD	77±16.23	6.09±0.78	167.3±52.99	64.3±14.07	0.39±0.15
5TD (13.5 mg/kg)					
n	10	10	10	10	10
Mean±SD	86.1±35.48	5.99±0.90*	150±58.57	51.3±8.81	0.35±0.15
TD (2.7 mg/kg)					
n	10	10	10	10	10
Mean±SD	65.2±19.83	5.81±1.25*	158±40.88	61.7±27.19	0.42±0.17

Figures in parenthesis indicate number of animals. *Significant at 5% level. SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, HD: High dose, PLT: Platelet, TD: Therapeutic dose

nontoxic character of mercury in *Arogyavardhini Vati* to process of *Shodhana* due to which, mercury may not retain its original physiochemical form and hence toxic character. According to an experimental toxicity study,[12] *Makaradhwaja* when given orally did not produced mortality up to the dose of 480 mg/kg in mice. A 28 days repeated dose oral toxicity study of *Siddha*

Table 9: Serum biochemical analysis of female rats at 90th day

Group	Glucose (mg/dl)	Total protein (g/dl)	SGOT (U/L)	SGPT (U/L)	Serum creatinine (mg/dl)
Control					
n	9	9	9	9	9
Mean±SD	73.78±13.99	6.29±0.47	143.44±38.18	47.89±7.01	0.48±0.21
HD (27.0 mg/kg)					
n	9	9	9	9	9
Mean±SD	80.78±27.19	6.38±0.56	155.89±64.58	45.22±11.17	0.38±0.21
5TD (13.5 mg/kg)					
n	9	9	9	9	9
Mean±SD	87.78±21.21	6.54±0.99	142.56±51.92	43.78±9.88	0.44±0.25
TD (2.7 mg/kg)					
n	10	10	10	10	10
Mean±SD	75.4±24.24	6.07±1.57	149±48.13	42.1±13.61	0.42±0.19

Figures in parenthesis indicate number of animals. SD: Standard deviation, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, HD: High dose, PLT: Platelet, TD: Therapeutic dose

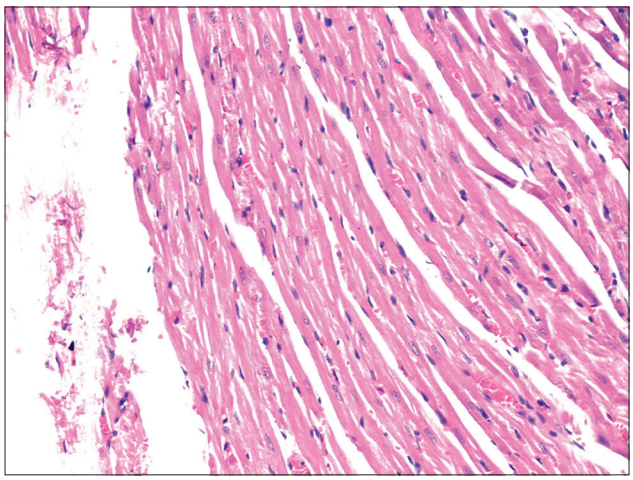

Figure 1: Heart control group: No abnormality detected (10 × 10)

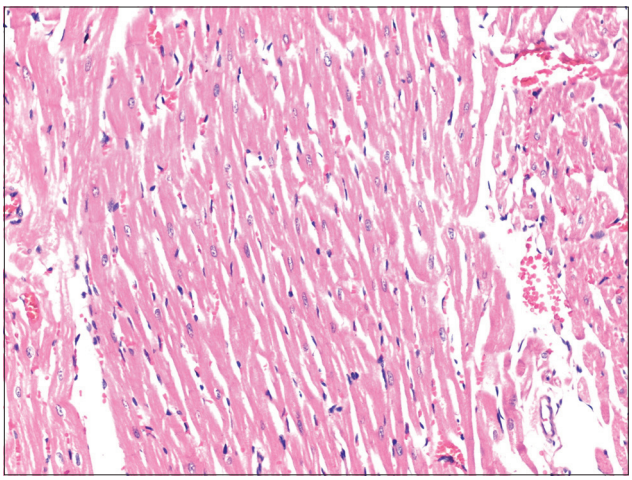

Figure 2: Heart high-dose group: No abnormality detected (10 × 10)

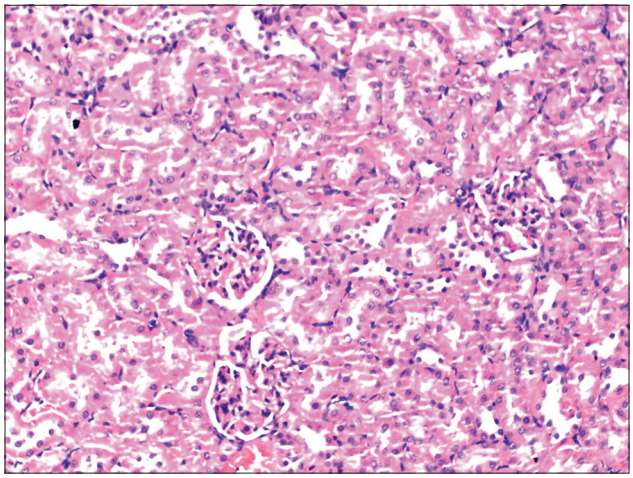

Figure 3: Kidney control group: No abnormality detected (10 × 10)

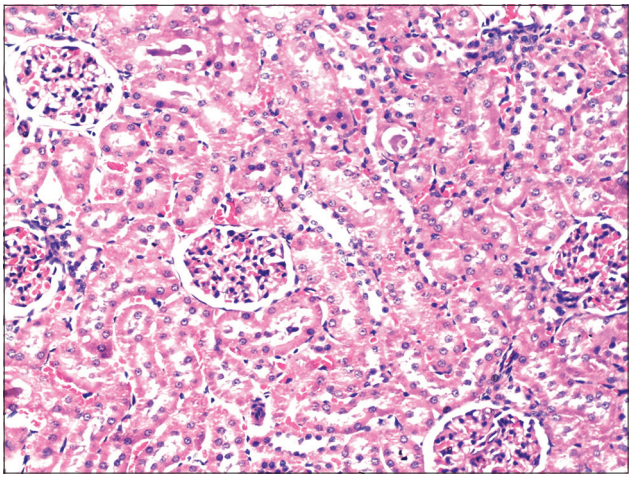

Figure 4: Kidney High Dose Group: No abnormality detected (10× 10×)

Makaradhwaja in rats showed neurodegenerative changes in brain at the dose of 100 mg/kg, but no histopathological changes were seen at dose level of 50 mg/kg in the kidney, liver and brain. Further no changes were seen in serum alanine aminotransferase, aspartate aminotransferase, alanine phosphatase, bilirubin, urea and creatinine implying the safety of *Siddha Makaradhwaja* on hepatorenal system of the rats.[13] Both *Triguna* and *Shadaguna Makaradhwaja*

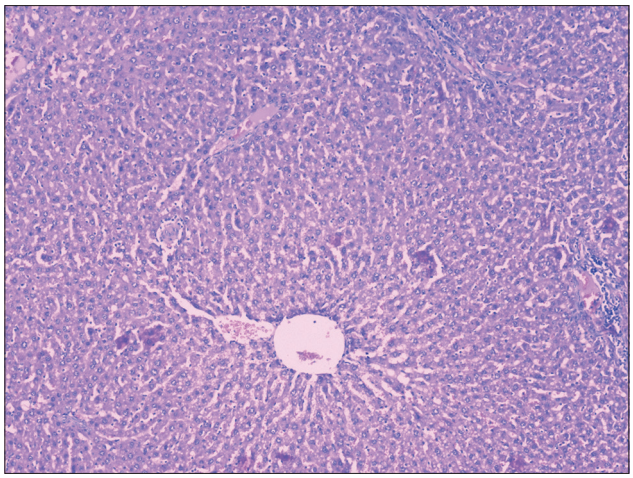

Figure 5: Liver control group: No abnormality detected (10 × 10)

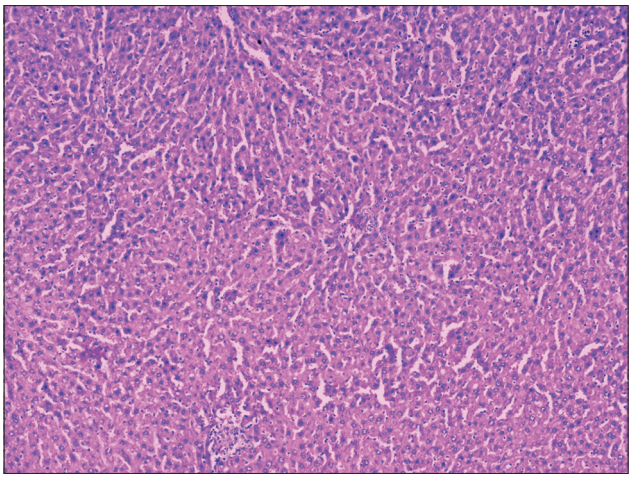

Figure 6: Liver high-dose group: No abnormality detected (10 × 10)

offered *Madhumehahara* property (antidiabetic) in clinical trials, but *Shadguna Balijarita* possessed more effect than *Triguna Balijarita Makaradhwaja*. It has experimentally shown antihyperglycemic, antidiabetic, renoprotective, cardiac stimulant activity and is a known immunomodulating agent in experimental studies with its use being reached in veterinary practices too.[14] A pilot clinical trial of *Makaradhwaja* has shown promising results for treating rheumatoid arthritis, but the study also showed mercury excretion in urine.[15] In the present study, the results are in line with earlier studies and histopathological evaluation also indicated no evidence of treatment-related or dose dependent lesions in any organ. It is apparent that *Makaradhwaja* is safe at dose rate of 27 mg/kg/day for ninety consecutive days. However, there was significant increase in kidney weights of females from HD group, which could not be corroborated with serum creatinine or other biochemical data and histopathological evaluation findings. Therefore, HD level, that is, 27 mg/kg body weight was decided as NOAEL and mid-dose level, that is, 13.5 mg/kg body weight was decided as NOEL was. Thus, the output of the study is in accordance with the results and conclusion by Kumar *et al*.[11] which underlined that the mere presence of very high proportion of mercury in *Makaradhawaja* does not possess any risk or toxic potential to animals or humans. The purification process adds several organic chemicals to the crude form of mercury which may lead to altered physicochemical properties of mercury and hence alters its toxic potential.[11,16-18]

Conclusion

When *Makaradhwaja* was administered daily for ninety consecutive days at dose levels 27, 13.5 and 2.7 mg/kg/day by oral route to Wistar rats, it was found that there was no effect on body weights, feed consumption and biochemical parameters. There was significant increase in kidney weight of male and females treated with HD which could not be correlated with histopathological and serum biochemical changes. There was no treatment-related changes observed in the histopathological evaluation at dose level 27 mg/kg. There was significant increase in MCV value of males treated with HD but was within biological limits. The dose level 27 mg/kg of *Makaradhwaja* was found as NOAEL and dose level 13.5 mg/kg of *Makaradhwaja* was found as NOEL. Ayurvedic preparation method of *Makaradhwaja* could be altering physicochemical properties of raw mercury rendering it to become significantly less harmful.

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Conflicts of interest

There are no conflicts of interest.

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