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Evaluation of Subchronic Toxicity of *Kataka Khadiradi Kasayam*in Wistar Albino Rats

Author: Sithara M S[1]

Co Authors: Joy Verghese T[2], Hariprasad P[3] andMigy John[4]

[1-4]Confederationfor Ayurvedic Renaissance – Keralam (CARe Keralam) Limited, KINFRA Small Industries Park, KINFRA Park P.O., Nalukettu Road, Koratty, Thrissur, Kerala, India

ABSTRACT

Background: *KatakakhadirādiKaṣāyaṃ*is a classical Ayurvedic Polyherbal preparation used for the treatment of diabetes, skin, and urinary tract ailments. However, to scientifically validate its efficacy and also to develop it as a product fulfilling all domestic and international regulatory guidelines, preclinical *in vivo* safety and efficacy data is essential.

Objectives: To assess the toxic potential of, *KatakakhadirādiKaṣāyaṃ,*when administered orally in repeated doses to Wistar rats.

Methods: *KatakakhadirādiKaṣāyaṃ*was manufactured at CARe Keralam Ltd. Subchronictoxicity study of *KatakakhadirādiKaṣāyaṃ***,** with 28 day reversal period, was conducted in six groups of rats with equal number of males and females. On completion of treatment and recovery periods, blood and tissue samples were collected from rats, from respective groups, for evaluation.

Results: Hematological and serum biochemical analysis data did not show any statistically significant differences in *KatakakhadirādiKaṣāyaṃ*treated groups compared to respective normal control groups. On necropsy, no gross pathological changes were noted in the treated animals compared to the control animals. Histopathological examination of internal organ samples from the control and the treatment groups did not reveal any pathologically significant changes.

Conclusions: From the results of this study, the No-Observed-Adverse-Effect–Level (NOAEL) of *KatakakhadirādiKaṣāyaṃ* in Wistar rats, after administration for 90 days was found to be 2300 mg/kg.

Key Words Subchronic**,** *Katakakhadirādi Kaṣāyaṃ, Toxicity, Polyherbal formulation, Wistar rats*

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INTRODUCTION

Polyherbal Ayurvedic formulations are the backbone of the Ayurvedic system of medicine due to their increased efficacy and negligible side effects as compared to the use of single herbs. But scientific validation, with regard to the quality, safety and efficacy, is very less in polyherbal Ayurvedic formulations when compared to single herbs. *Katakakhadirādi Kaṣāyaṃ* is such a classical Ayurvedic polyherbal preparation mentioned in Sahasrayogam[1], one of the most important classics in Ayurveda. *Katakakhadirādi Kaṣāyaṃ* is used for the treatment of diabetes, skin, and

ORIGINAL RESEARCH ARTICLE

urinary tract ailments and also controls both Vata and Kapha related ailments[2]. *KatakakhadirādiKaṣāyaṃ*has been reported to have antioxidant property, which could be considered as a major step in understanding its scientific role in treating diabetes[3]. However, further parameters need to be verified to prove the efficacy of this formulation as an antidiabetic. First of all, to validate its efficacy and also to develop it as a product fulfilling all domestic and international regulatory guidelines, preclinical *in vivo* safety and efficacy data is essential. Hence, the objective of this study was to evaluate the possible health hazards likely to arise from repeated exposure of *KatakakhadirādiKaṣāyaṃ*in laboratory ratsover a relatively limited period.

MATERIALS AND METHODS

Preparation of *KatakakhadirādiKaṣāyaṃ*

The test item, *KatakakhadirādiKaṣāyaṃ***,** a brown-colored suspension, was manufactured at CARe Keralam Ltd.

Equal quantity of raw materials of Pharmacopeial quality viz.,*Kataka (Strychnospotatorum)* (Seed), *Khadira (Acacia catechu)* (Heartwood), *Dhatri (Emblicaofficinalis)* (Pericarp), *Vairi (Salaciareticulata)* (Root), *Darvi (Berberisaristata)* (Stem), *Samanga (Biophytumsensitivum)* (Whole Plant), *Rajani (Curcuma longa)* (Rhizome), *Pata (Cycleapeltata)* (Rhizome),*Chootabija – (Mangiferaindica)* (Seed), *Abhaya (Terminalia chebula)* (Pericarp), *Abda (Cyperusrotundus)* (Rhizome) and *Kola (Zizyphus jujube)* (Seed),were boiled in 16 parts of fresh water and the volume was reduced to one fourth. Then it was filtered and again concentrated to 1/4th quantity and preserved using 0.1 % of Sodium benzoatesolution.

Ethics statement

The study was performed following the standard operating procedures at CARe KERALAM Ltd. and the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, January 7th, 2010. The protocols were approved by Institutional Animal Ethics Committee (IAEC), with protocol no. CKL/TOX/IAEC/025-14.

Experimental animals

Wistar albino rats(8 to 12 Weeks age) of both sexes were utilized in this study. Animals were housed under standard laboratory conditions(22 ± 3ºC room temperature and 50-60% humidity):air-conditioned environment with adequate fresh air supplywith Individually Ventilated Caging (IVC) system (Air changes 15/hr), with12 hrslight and 12 hrs dark cycle. The temperature and relative humidity were recorded daily. The animals were acclimatized for a minimum period of seven days to laboratory conditions before the initiation of the experiment.The animals were fed *ad libitum*and water was provided *ad libitum* throughout the acclimatization and study period.

Experimental design

ORIGINAL RESEARCH ARTICLE

Dose levels selected for the repeated dose study were based upon the maximum human dose used in clinical practice. The test was conducted based upon OECD Guideline No. 408[4].Six groups consisting of equal male and female rats were maintained in the study. Group-I. II, III, IV, V, and VI served as control, control recovery, low, mid, high dose, and high dose recovery groups respectively. Animals were treated in the following manner;

- **GroupI (Normal control)** – 6 animals (3 male and 3 female) receive 1 ml/100g distilled water, orally
- **Group II (Normal control recovery)** – 6 animals (3 male and 3 female) receive 1 ml/100g distilled water, per orally
- **Group III (Low dose)** – 10 animals (5 male and 5 female) receive ***KatakakhadirādiKaṣāyaṃ***575 mg/kg, per orally
- **Group IV (Mid dose)** – 10 animals (5 male and 5 female) receive ***KatakakhadirādiKaṣāyaṃ***1150 mg/kg, per orally
- **Group V (High dose)** – 10 animals (5 male and 5 female) receive ***KatakakhadirādiKaṣāyaṃ***2300 mg/kg, per orally
- **Group VI (High dose recovery)** – 10 animals (5 male and 5 female) receive ***KatakakhadirādiKaṣāyaṃ***2300 mg/kg, per orally

The test item was administered through oral route for 90 consecutive days to all treatment groups. The animals in recovery groups were kept for another 28 days for observation.

All animals were observed daily in the morning and again in the afternoon for clinical signs and mortality during the study period. The body weight of each rat was recorded before treatment on day 1, weekly thereafter, and at the last day of treatment. Percent body weight gain was calculated for each group on weekly basis. The quantity of food and water consumed by rats in each cage was measured and recorded from the day of commencement of treatment and average weekly consumption was calculated.

At the end of treatment on the 91st day, all surviving animals ingroups I, III, IV, V, and on the 120thday animals in groups II and VIwere fasted overnight. *Ad libitum* water was given during fasting. Blood samples were collected from the orbital plexus, under anesthesia, with EDTA anticoagulant for determining the hematological parameters like Haemoglobin (Hb), Erythrocyte Count (Total RBC), Leukocyte Count (Total WBC), and Platelet Count.The serum was separated by centrifuging the blood samples, collected in plain tubes (without EDTA), at 3000 rpm for 10 min for determining the clinical chemistry parameters, such as Glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lipid profile, Total Protein (TP), Albumin,Bilirubin, Creatinine, and Urea. All animals were then sacrificed by cervical dislocation. Gross examination of carcasses was conducted. The representative tissue samples of organswere collected and preserved in 10% neutral buffered formalin. The tissues were

ORIGINAL RESEARCH ARTICLE

embedded in paraffin wax, sectioned at five micrometers, and stained with haematoxylin and eosin. A detailed histopathological examination was performed.

Statistical Analysis

All data has been represented as mean ± SD. The data on bodyweight, food intake, water intake, hematology, clinical chemistry generated from the present study were subjected to computer statistical analysis using GraphPad Prism software, Version 5.00, USA. 2007.

One-way ANOVA with Dunnets post-test was done for different treatment groups comparing with the Control group data. The unpaired't'-test was done for recovery control and high dose recovery group data. All analysis and comparisons were evaluated at 5% significance level.P< 0.05 was accepted as statistically significant. P < 0.05, P < 0.01 and P < 0.001 are represented by *, **, and *** respectively.

RESULTS AND DISCUSSION

Clinical signs and pre-terminal deaths were not observed in any of the groups tested with *KatakakhadirādiKaṣāyaṃ*. In the present study, none of the animals in treatment groups showed any statistically significant variations in body weights compared to the respective control group indicating that the test item did not have any effect on body weights. Generally, body weight changes are an indicator of adverse side effects, after exposure to toxic substances. The animals that survived cannot lose more than 10% of the initial body weight [5]. Any variations in body weight can be regarded as a rapid assessment of the side effects of a drug[6]. In the present study, none of the animals in treatment groups showed any statistically significant variations in body weights compared to the respective control group indicating that the test item did not have any effect on body weights (Fig – 1, 2 & 3).Bodyweight gain of *KatakakhadirādiKaṣāyaṃ*treated rats of both sexes was comparable to that of respective control group rats (Table – 1, 2 & 3).

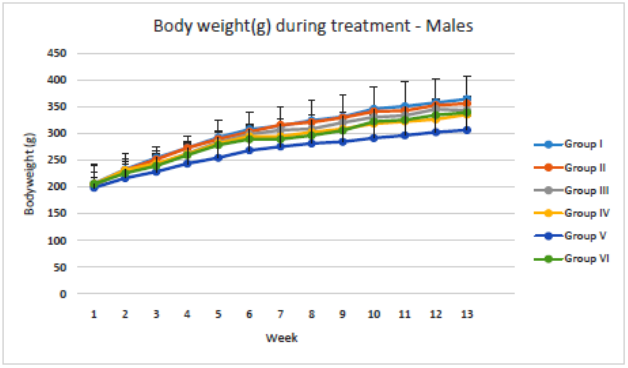

Figure 1. Body weight (g) of males during treatment period

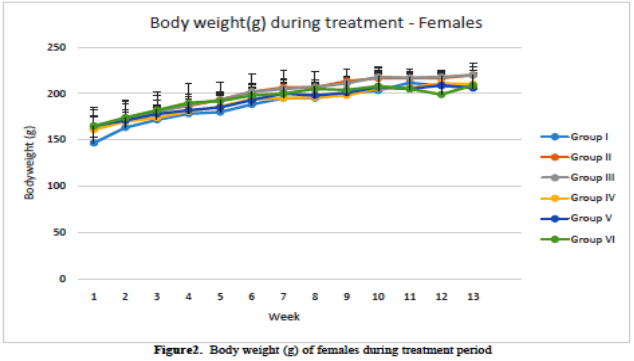

Figure2. Body weight (g) of females during treatment period

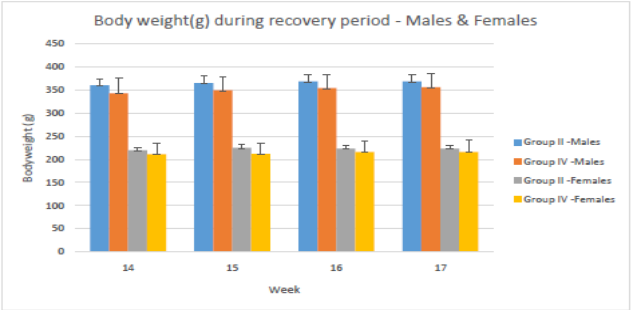

Figure 3. Body weight (g) of animals during recovery period

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Table 1 Percent bodyweight gain of Males during treatment period

Groups	WEEK											
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Group I	12.88	9.19	7.20	7.18	5.15	2.12	3.16	2.01	4.57	1.35	1.92	1.94
	±	±	±	±	±	±	±	±	±	±	±	±
	0.66	2.69	1.11	2.44	3.67	0.68	1.61	0.64	0.54	1.29	0.87	1.07
Group II	13.98	8.64	8.58	6.14	4.69	4.42	1.57	2.61	3.57	0.48	2.90	0.97
	±	±	±	±	±	±	±	±	±	±	±	±
	1.83	0.22	2.89	4.59	4.36	1.09	1.54	0.94	1.82	0.84	2.50	2.14
Group III	12.66	5.73	9.11	8.02	5.28	2.73	0.68	3.88	3.12	0.91	3.61	-0.58
	±	±	±	±	±	±	±	±	±	±	±	±
	4.88	5.23	7.11	1.14	2.71	1.93	1.86	2.91	1.06	1.38	0.92	1.28
Group IV	13.18	5.60	7.55	7.66	4.63	0.06	2.79	2.03	3.39	1.16	1.21	2.90
	±	±	±	±	±	±	±	±	±	±	±	±
	4.55	4.21	4.07	2.37	2.32	4.37	3.78	1.50	2.83	2.26	0.68	2.78
Group V	9.68	5.67	6.94	4.79	5.58	2.56	2.25	1.14	2.58	1.70	2.09	1.35
	±	±	±	±	±	±	±	±	±	±	±	±
	4.16	4.69	4.75	2.88	2.73	2.97	2.33	2.05	3.05	1.10	3.02	0.77
Group VI	10.13	6.18	8.45	7.28	4.16	0.10	2.43	2.99	5.50	0.92	2.78	1.44
	±	±	±	±	±	±	±	±	±	±	±	±
	6.55	3.62	5.17	2.30	4.31	3.94	1.91	2.09	2.43	1.41	1.29	2.41

Values are expressed as mean ± SD

Table 2 Percent bodyweight gain of Females during treatment period

Groups	**WEEK**											
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Group I	11.27	5.27	3.94	1.01	4.72	3.49	0.00	2.57	1.57	4.34	-1.63	-0.83
	±	±	±	±	±	±	±	±	±	±	±	±
	10.62	2.23	1.85	1.75	1.87	1.31	0.00	0.17	3.72	4.01	1.41	1.44
Group II	7.40	4.96	2.89	3.76	4.30	2.48	0.06	3.21	1.55	0.02	0.02	1.59
	±	±	±	±	±	±	±	±	±	±	±	±
	2.60	2.08	2.86	4.38	1.45	0.09	2.41	1.34	1.34	2.35	2.35	2.75
Group III	7.67	3.50	4.47	2.70	4.68	1.49	0.97	2.00	3.31	-0.36	0.52	0.91
	±	±	±	±	±	±	±	±	±	±	±	±
	4.31	1.54	1.58	2.71	2.23	2.20	1.32	2.61	3.62	3.64	2.95	1.25
Group IV	5.90	2.34	3.91	2.82	4.28	0.51	0.63	1.08	4.08	-0.01	2.89	-0.52
	±	±	±	±	±	±	±	±	±	±	±	±
	4.45	2.51	2.48	3.32	1.37	2.07	3.32	1.48	3.85	1.63	7.76	7.93
Group V	3.81	4.31	2.27	1.65	4.44	3.58	-0.91	1.45	2.97	-0.89	1.98	-1.44
	±	±	±	±	±	±	±	±	±	±	±	±
	2.62	2.85	1.29	1.53	1.78	2.34	2.36	2.93	0.96	2.63	1.12	1.33
Group VI	5.71	4.57	4.38	1.13	3.01	0.98	2.84	-0.59	2.03	-1.45	-2.75	5.23
	±	±	±	±	±	±	±	±	±	±	±	±
	3.88	2.33	1.43	1.56	1.97	2.42	3.99	2.71	2.23	2.25	6.68	8.16

Values are expressed as mean ± SD

Table 3 Percent Body Weight Gain of Males and Females during Recovery Period

Group	**Sex**	**WEEK**			
		14	**15**	**16**	**17**
Group II	Male	0.92	1.38	0.91	0.00
		±	±	±	±
		0.80	1.35	1.58	0.00
	Female	0.00	2.26	-0.71	0.00
		±	±	±	±
		0.00	2.22	1.23	0.00
Group VI	Male	0.86	1.81	1.45	0.59

Int J Ayu Pharm Chem
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ORIGINAL RESEARCH ARTICLE

	± 0.80	± 1.31	± 1.11	± 0.81
Female	0.94 ± 2.59	0.56 ± 1.90	1.89 ± 2.92	-0.07 ± 1.70

Values are expressed as mean ± SD

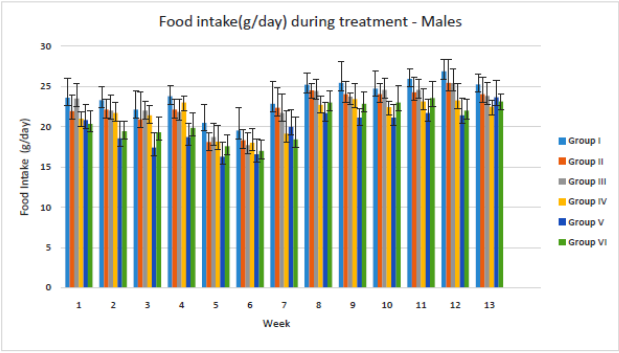

Figure 4. Average Food intake (g/day) of males during treatment period

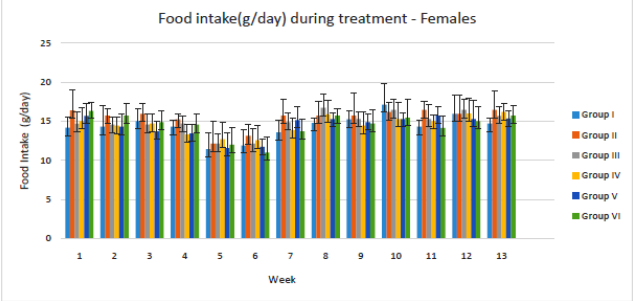

Figure 5. Average Food intake (g/day) of females during treatment period

A slight reduction in the food consumption of males and females was noted during the treatment period. However, there was no statistically significant difference noted in the average weekly food consumption of animals in treatment and recovery groups compared to the respective control groups (Fig – 4, 5 & 6). Bodyweight gain and feed consumption are said to be a non-specific,broad screen for adverse systemic toxicity[7]. Theconsistent observation of normal pattern in body weight gain and feed consumptionof *KatakakhadirādiKaṣāyaṃ*treated rats of both sexesthroughout the study period suggested normalgrowth and development pattern.

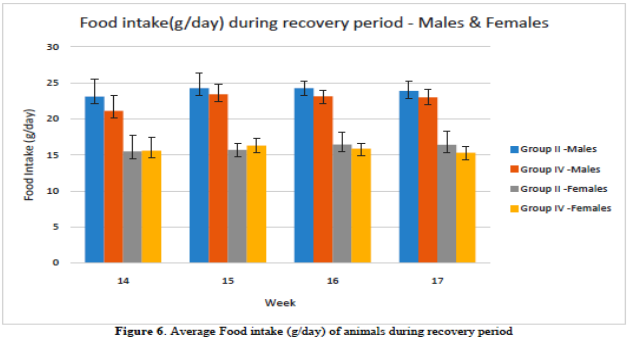

Figure 6. Average Food intake (g/day) of animals during recovery period

Table 4 Average Weekly Water Intake (ml) of Males during Treatment Period

Groups	Week												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Group I	49.50 ± 3.69	46.88 ± 6.03	45.91 ± 4.01	43.05 ± 2.93	23.05 ± 8.72	27.38 ± 7.60	48.43 ± 2.93	44.81 ± 4.17	42.52 ± 5.50	45.48 ± 3.73	47.81 ± 6.23	43.19 ± 3.48	48.00 ± 1.92
Group II	44.78 ± 3.54	42.38 ± 3.99	39.67** ± 2.65	35.43*** ± 2.54	18.24 ± 7.00	24.00 ± 7.36	39.48** ± 5.58	39.86 ± 5.58	40.33 ± 4.90	43.52 ± 6.43	43.10 ± 5.28	37.95 ± 5.38	40.00 ± 4.88
Group III	49.73 ± 1.96	45.14 ± 5.30	45.51 ± 3.96	38.74 ± 3.80	31.77 ± 3.21	24.63 ± 6.45	48.40 ± 3.98	47.14 ± 3.66	45.83 ± 5.67	43.80 ± 4.94	46.20 ± 4.04	46.83 ± 2.12	43.50 ± 7.05
Group IV	44.00 ± 3.29	40.14 ± 3.47	40.40 ± 2.01	35.69*** ± 2.01	28.81 ± 1.75	24.37 ± 4.18	38.20** ± 5.16	44.11 ± 4.10	43.17 ± 5.13	41.00 ± 4.34	43.54 ± 2.41	39.26 ± 3.05	40.83 ± 2.94
Group V	44.30	36.31***	39.26**	34.54***	25.09	22.51	39.63**	38.51*	40.34	36.60**	42.34	36.03	39.03**

ORIGINAL RESEARCH ARTICLE

	±	±	±	±	±	±	±	±	±	±	±	±	±
	3.43	3.10	1.63	2.47	1.98	3.72	4.03	3.24	2.92	3.01	3.13	5.94	3.88
	45.00	40.77	40.97	42.29	27.49	23.09	37.51***	42.51	41.91	39.83	41.23	44.29	42.47
Group VI	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.54	4.74	4.02	2.19	1.91	4.94	5.70	3.25	4.38	2.12	3.43	3.87	2.47

Values are expressed as mean ± SD*P<0.05, ** P<0.01, *** P<0.001 when compared with Group I

Table 5 Average Weekly Water Intake (ml) of Females during Treatment Period

Groups	Week												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Group I	31.000 ± 4.624	26.619 ± 3.937	30.429 ± 3.446	25.429 ± 1.893	17.095 ± 6.760	21.381 ± 6.657	32.857 ± 4.472	28.524 ± 5.567	31.286 ± 4.596	31.381 ± 4.767	35.524 ± 5.962	31.000 ± 5.796	35.944 ± 8.169
Group II	31.444 ± 6.306	33.214 ± 3.879	32.095 ± 4.909	27.714 ± 0.989	17.952 ± 8.665	23.238 ± 4.446	33.238 ± 4.965	32.333 ± 5.358	38.952 ± 5.522	35.286 ± 3.817	40.095 ± 2.699	35.524 ± 4.985	37.762 ± 4.353
Group III	28.067 ± 2.744	33.000 ± 6.038	30.314 ± 3.160	22.429 ± 3.265	22.514 ± 0.915	22.029 ± 2.544	33.686 ± 2.635	32.514 ± 2.463	36.200 ± 3.499	31.114 ± 6.518	34.457 ± 2.571	30.286 ± 5.545	31.067 ± 4.283
Group IV	32.600 ± 4.571	27.543 ± 2.759	29.371 ± 1.560	27.514 ± 1.911	23.714 ± 1.311	21.971 ± 3.069	32.743 ± 3.553	34.314 ± 2.625	38.543 ± 4.233	34.429 ± 1.734	33.143 ± 2.930	37.000 ± 6.105	33.900 ± 5.070
Group V	32.800 ± 2.539	33.379 ± 5.103	33.857 ± 1.539	30.743 ± 3.006	23.343 ± 1.924	22.657 ± 3.115	36.829 ± 2.213	31.200 ± 6.074	36.693 ± 3.053	28.371 ± 3.966	37.286 ± 2.759	38.629 ± 4.320	38.500 ± 5.149
Group VI	30.900 ± 4.027	30.629 ± 4.438	28.657 ± 1.750	29.629 ± 2.328	22.800 ± 2.422	19.971 ± 3.641	27.457 ± 2.065	30.086 ± 5.512	33.343 ± 3.612	27.800 ± 4.376	34.800 ± 3.767	31.429 ± 8.607	36.314 ± 6.119

Values are expressed as mean ± SD

Table 6 Average Weekly Water Consumption (ml) of Males and Females during Recovery Period

Group	Sex	WEEK			
		14	**15**	**16**	**17**
Group II	Male	32.714 ± 4.062	34.095 ± 2.685	40.857 ± 5.517	36.722 ± 2.205
	Female	30.000 ± 3.825	28.571 ± 2.250	33.857 ± 4.713	27.444 ± 2.062
Group VI	Male	41.829 ± 1.951	37.571 ± 4.982	39.029 ± 4.492	38.200 ± 3.303
	Female	29.486 ± 3.139	29.657 ± 1.735	30.943 ± 5.564	30.200 ± 4.218

Values are expressed as mean ± SD

A significant reduction in the average weekly water intake of males was observed in treatment groups except in the 1st, 5th, 6th, 9th, 11th, and 12th weeks. But this reduction in water intake was also noted in the control recovery group at 3rd, 4th, and 7th weeks. Hence, it can be considered as an individual animal variation or a temporary change, as during the recovery period there was

ORIGINAL RESEARCH ARTICLE

no significant change in the water consumption in rats treated compared to that of the control group. However, no significant clinical signs or changes in activity related to the administration of *KatakakhadirādiKaṣāyaṃ*were observed in these rats. Therefore, the change could not be regarded as toxicologically significant. Regarding the average weekly water intake of females during treatment and recovery periods, all the values were comparable to normal control in all the groups (Table - 4, 5 & 6). Bodyweight, food consumption, and water intake are interrelated and are the main indicators of adverse effects of a toxic substance in rodents[8, 9]. Any variation in food and water consumption will directly affect the normal metabolism of animals[10].

The hemopoietic system is considered to be the primary target of many xenobiotics and is a sensitive marker for pathological conditions[11]. In the present study, *KatakakhadirādiKaṣāyaṃ*treatment did not result in any adverse effect on hematological parameters estimated in both the sexes of treatment and recovery groups when compared with control (Tables – 7, 8 & 9).

Table 7 Hematology Parameters of Males in Treatment Groups

Group	Total Erythrocyte count (10^6 cells/µl)	Hb (g/dl)	Total Leucocyte count (10^3 cells/µl)	Platelet count (10^3 cells/µl)
Group I	8.363 ± 0.217	15.767 ± 0.404	8.533 ± 1.060	658.667 ± 56.757
Group II	7.758 ± 0.160	14.640 ± 0.207	9.040 ± 0.472	821.600 ± 67.441
Group III	7.724 ± 0.466	14.760 ± 0.915	9.540 ± 0.764	669.000 ± 188.413
Group IV	7.704 ± 0.248	14.620 ± 0.303	9.500 ± 0.640	758.800 ± 12.194

Values are expressed as mean ± SD

Table 8 Hematology of Females in Treatment Groups

Group	Total Erythrocyte count (10^6 cells/µl)	Hb (g/dl)	Total Leucocyte count (10^3 cells/µl)	Platelet count (10^3 cells/µl)
Group I	7.550 ± 0.537	16.033 ± 2.021	5.033 ± 0.586	578.333 ± 47.385
Group II	6.674 ± 0.990	13.240 ± 1.913	8.660 ± 2.454	674.400 ± 129.984
Group III	7.366 ± 0.318	14.540 ± 0.385	8.120 ± 2.238	769.800 ± 48.638
Group IV	7.456 ± 0.351	14.740 ± 0.882	8.020 ± 1.363	740.200 ± 169.462

Values are expressed as mean ± SD

Table 9 Hematology of Males and Females in Recovery Groups

Group	Sex	Total Erythrocyte count (10^6 cells/µl)	Hb (g/dl)	Total Leucocyte count (10^3 cells/µl)	Platelet count (10^3 cells/µl)
Group II	Male	8.170 ± 0.386	15.033 ± 0.451	8.333 ± 1.115	705.000 ± 217.000
	Female	7.690 ± 0.052	14.967 ± 0.551	7.300 ± 1.400	686.333 ± 230.405
Group VI	Male	8.084 ± 0.407	15.200 ± 0.675	8.120 ± 0.672	643.200 ± 141.992
	Female	7.768 ± 0.392	15.520 ± 0.719	5.720 ± 0.653	644.000 ± 94.003

Values are expressed as mean ± SD

Blood parameters analysis is relevant to risk evaluation as the hematological system has a higherpredictive value for toxicity in humans (91%)[12]. Being a transport medium the blood carries many drugs and xenobiotics due to which the components of the blood such as red blood cells, white blood cells, hemoglobin, and platelets are forced to initially expose to significant concentrations of the toxic compound. Any damage and destruction of the blood components will affect the normal functioning of the body. However, in the present investigation,

ORIGINAL RESEARCH ARTICLE

*KatakakhadirādiKaṣāyaṃ*did not bring about any significant effect on the hematological parameters measured,suggesting the nontoxic nature of the formulation.

Serum biochemical studies could provide a significant inference about the nature of toxic effects on the liver[13]. Hepatic toxicity is regarded as one of the common side effects of several clinically used agents leading to restricted use or even withdrawal of drug[14, 15]. The enzymes AST, ALT, ALP, and the biochemical parameter bilirubin are considered as the markers of liver function[16]. Hepatocellular damage is characterized by the rise in serum levels of both AST and ALT. But since ALT is located mainly in the cytoplasm of hepatocytes, this enzyme is a more sensitive marker of hepatocellular damage than AST and within limits can provide a quantitative assessment of the degree of damage sustained by the liver[17]. However, oral administration of *KatakakhadirādiKaṣāyaṃ*did not result in elevation of any of these enzyme levels in all treatment groups. All Clinical chemistry parameters of *KatakakhadirādiKaṣāyaṃ*treatment and recovery group animals were comparable with that of the respective control group animals.Kidney function was evaluated using serum urea and creatinine concentrations. Serum urea and creatinine levels in treated groups were also comparable to normal control groups. In preclinical toxicity studies, renal changes are more likely to occur because of the high doses given and the fact that the kidneys eliminate many drugs and their metabolites[18, 19]. However, repeated oral administration of *KatakakhadirādiKaṣāyaṃ*for 90 days did not cause any significant differences in serum biochemical values of any of the study animals. These results thus indicate that the *KatakakhadirādiKaṣāyaṃ*does not harm blood and serum parameters at a dose up to 2300 mg/kg (Tables – 10, 11 & 12).

On gross examination, any pathologically relevant lesions were not detected in any of the organs. There is a very high possibility that some of the herbal drugs may cause severe toxicity to

Table 10 Serum Biochemistry of Males in Treatment Groups

Parameters	Group I	Group III	Group IV	Group V
Total Cholesterol (mg/dl)	78.60 ± 16.37	79.62 ± 11.19	80.20 ± 8.71	63.90 ± 11.83
Triglycerides (mg/dl)	67.30 ± 3.42	96.06 ± 14.64	102.06 ± 30.97	87.34 ± 16.12
High Density Lipoprotein (HDL) (mg/dl)	56.20 ± 11.62	52.12 ± 10.74	48.86 ± 8.85	42.38 ± 4.62
AST (U/L)	183.20 ± 37.41	203.42 ± 23.55	234.24 ± 45.86	217.22 ± 50.69
ALT (U/L)	50.33 ± 2.80	59.72 ± 7.29	58.36 ± 5.56	53.24 ± 7.11
ALP (U/L)	282.30 ± 151.95	391.08 ± 137.28	270.98 ± 88.68	267.40 ± 64.61
Glucose (mg/dl)	97.73 ± 6.22	101.12 ± 19.81	108.16 ± 15.18	102.08 ± 13.66
TP (g/dl)	6.73 ± 0.72	6.88 ± 0.61	7.39 ± 0.96	6.47 ± 0.85
Albumin (g/dl)	2.00 ± 0.10	2.06 ± 0.06	2.14 ± 0.22	1.90 ± 0.30
Bilirubin Total (mg/dl)	0.13 ± 0.06	0.10 ± 0.12	0.10 ± 0.07	0.12 ± 0.11
Bilirubin Direct (mg/dl)	0.03 ± 0.06	0.04 ± 0.09	0.00 ± 0.00	0.00 ± 0.00
Uric acid (mg/dl)	2.66 ± 0.52	2.14 ± 0.57	2.03 ± 0.36	2.66 ± 0.76
Urea (mg/dl)	34.83 ± 1.31	37.74 ± 5.00	30.04 ± 17.21	37.96 ± 3.90
Creatinine (mg/dl)	0.41 ± 0.05	0.45 ± 0.01	0.50 ± 0.01	0.51 ± 0.01

Values are expressed as mean ± SD

ORIGINAL RESEARCH ARTICLE

Table 11 Serum Biochemistry of Females in Treatment Groups

Parameters	Group I	Group III	Group IV	Group V
Total Cholesterol (mg/dl)	102.00 ± 1.92	100.38 ± 17.61	102.60 ± 6.23	100.66 ± 7.39
Triglycerides (mg/dl)	89.53 ± 14.72	131.46 ± 30.12	93.58 ± 13.59	98.10 ± 15.38
High Density Lipoprotein (HDL) (mg/dl)	88.87 ± 1.64	77.36 ± 11.81	84.82 ± 4.40	78.40 ± 4.95
AST (U/L)	161.67 ± 18.05	175.12 ± 31.10	162.74 ± 30.52	166.00 ± 30.24
ALT (U/L)	43.13 ± 4.74	50.64 ± 0.69	48.32 ± 8.82	42.06 ± 2.60
ALP (U/L)	220.94 ± 38.86	231.08 ± 65.52	256.62 ± 35.21	214.94 ± 24.59
Glucose (mg/dl)	119.13 ± 13.05	90.080 ± 6.956	89.74 ± 13.99	119.12 ± 33.07
TP (g/dl)	7.51 ± 1.37	7.06 ± 0.43	6.73 ± 0.15	7.00 ± 0.81
Albumin (g/dl)	2.20 ± 0.27	2.02 ± 0.13	2.02 ± 0.05	2.02 ± 0.15
Bilirubin Total (mg/dl)	0.10 ± 0.00	0.18 ± 0.08	0.18 ± 0.05	0.14 ± 0.06
Bilirubin Direct (mg/dl)	0.10 ± 0.00	0.08 ± 0.05	0.02 ± 0.05	0.02 ± 0.05
Uric acid (mg/dl)	3.19 ± 0.49	3.32 ± 0.36	3.24 ± 0.52	3.77 ± 0.32
Urea (mg/dl)	31.33 ± 0.93	38.28 ± 3.67	37.60 ± 3.63	37.06 ± 2.81
Creatinine (mg/dl)	0.46 ± 0.01	0.48 ± 0.06	0.47 ± 0.07	0.50 ± 0.09

Values are expressed as mean ± SD

Table 12 Serum Biochemistry of Males and Females in Recovery Groups

Parameters	Group II		Group VI	
	Male	Female	Male	Female
Total Cholesterol (mg/dl)	96.90 ± 6.33	89.00 ± 5.43	96.50 ± 8.70	96.14 ± 18.72
Triglycerides (mg/dl)	75.43 ± 8.06	60.43 ± 3.77	76.70 ± 7.94	77.64 ± 10.33
High Density Lipoprotein (HDL) (mg/dl)	67.03 ± 12.12	72.50 ± 6.10	70.38 ± 17.21	77.68 ± 14.72
AST (U/L)	226.77 ± 14.40	225.73 ± 5.26	232.54 ± 33.65	224.26 ± 37.11
ALT (U/L)	51.50 ± 2.79	43.83 ± 1.42	57.56 ± 5.05	60.60 ± 4.59
ALP (U/L)	201.90 ± 77.64	200.53 ± 23.55	273.38 ± 81.74	181.54 ± 94.51
Glucose (mg/dl)	100.33 ± 10.44	90.80 ± 5.05	105.02 ± 12.22	93.46 ± 7.57
TP (g/dl)	7.82 ± 0.54	7.38 ± 0.76	7.18 ± 1.29	7.26 ± 0.54
Albumin (g/dl)	2.13 ± 0.21	2.10 ± 0.17	2.02 ± 0.19	2.04 ± 0.09
Bilirubin Total (mg/dl)	0.10 ± 0.00	0.17 ± 0.06	0.18 ± 0.13	0.18 ± 0.11
Bilirubin Direct (mg/dl)	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.05	0.06 ± 0.09
Uric acid (mg/dl)	2.17 ± 0.39	3.82 ± 2.72	2.58 ± 0.50	5.73 ± 2.99
Urea (mg/dl)	36.87 ± 2.37	32.07 ± 1.46	38.54 ± 3.31	39.14 ± 2.47
Creatinine (mg/dl)	0.39 ± 0.10	0.37 ± 0.12	0.42 ± 0.12	0.422 ± 0.14

Values are expressed as mean ± SD

the vital organs such as the kidney, liver, spleen, brain, heart, and lungs because of their diverse roles in the human body. The evaluation of histopathological changes in internal organs remains a cornerstone in the safety assessment of medicines[19]. In the present study, none of the animals in treatment and recovery groups showed any pathological changes in vital organs during evaluation, indicating that the administration of *KatakakhadirādiKaṣāyaṃ*did not cause any histological changes (Fig. 7 & 8).

ORIGINAL RESEARCH ARTICLE

CONCLUSION

In the current investigation, no clinical signs and mortality were noticed up to the high dose of 2300mg/kg. Any significant treatment related differences in body weight and body weight gains of animals across different groups and any

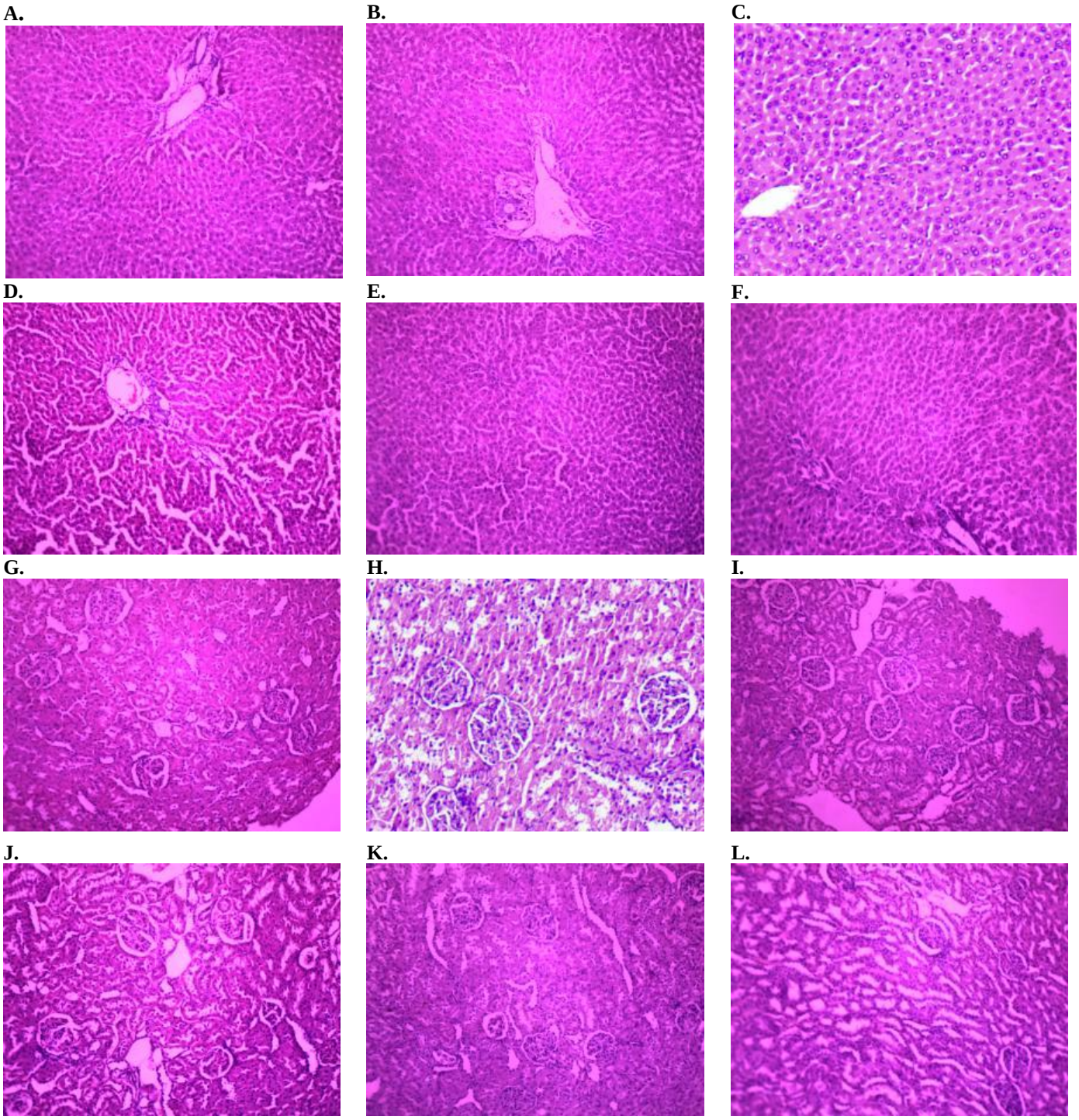

Figure 7. Histopathology of Liver and kidney in males from different groups. Photomicrographs showing normal cytoarchitecture of liver - A. Group I (Normal control), B. Group II (Recovery control), C. Group III, D. Group IV, E. Group V and F. Group VI. Photomicrographs showing normal cellular architecture of Kidney in males - G. Group I (Normal control), H. Group II (Recovery control), I. Group III, J. Group IV, K. Group V and L. Group VI. (H&E).

ORIGINAL RESEARCH ARTICLE

toxicologically significant effect on average daily food and water consumption were not observed in both the sexes up to 2300 mg/kg. No effect.onhematological and serum biochemical parameters of male and female rats treated up to the dose of 2300 mg/kg, were noted. No gross and microscopic pathological changes were noted in

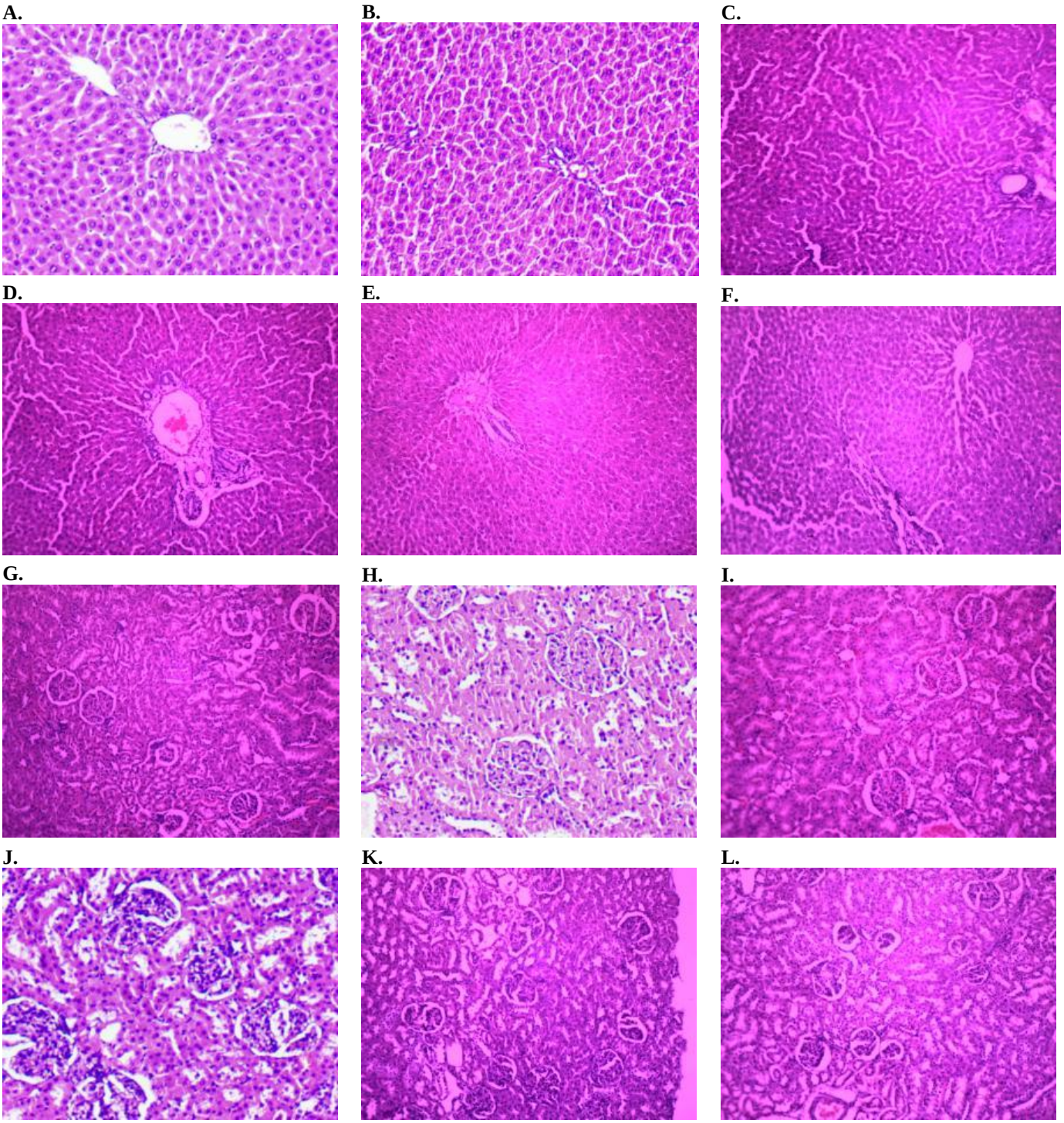

Figure 8. Histopathology of Liver and kidney in females from different groups. Photomicrographs showing normal cytoarchitecture of liver - A. Group I (Normal control), B. Group II (Recovery control), C. Group III, D. Group IV, E. Group V and F. Group VI. Photomicrographs showing normal cellular architecture of Kidney in females - G. Group I (Normal control), H. Group II (Recovery control), I. Group III, J. Group IV, K. Group V and L. Group VI. (H&E).

the vital tissues of male and female rats treated at and up to the level of 2300 mg/kg.

Based on the above findings, the No-Observed-Adverse -Effect –Level (NOAEL) of *KatakakhadirādiKaṣāyaṃ*in Wistar rats following the oral route of administration for 90 days was found to be 2300mg/kg.

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ORIGINAL RESEARCH ARTICLE

REFERENCES

1. K.V.Krishnanvaidyan& S. Gopala Pillai. (2006).Sahasrayogam with SujanapriyaVyakhya, 26th Ed. Mullakal: Vidyarambham Publishers; p.94.

2. AngelieJessica, S., Rao, M.R.K.,Anthony, J., Prabhu, K., Johnson, W.M.S., Balasubramanian, B.S., Sundaram, R. L. &Dinakar, S. (2016). The GC-MS Study of One Ayurvedic Preparation *KatakakhadiradiKashayam*.International Journal of Pharmaceutical Sciences Review and Research, 39(2), 216-224.

3. AngelieJessica, S., Rao, M.R.K., Anthony, J., Prabhu, K., Kavimani, M., Balasubramanian, B. S., Sundaram, R.L. &Dinakar, S. (2017). Antioxidant Study of One Ayurvedic Preparation *KatakakhadiradiKashayam*. Journal of Pharmaceutical Sciences and Research, 9(9), 1427-1429.

4. OECD (The Organisation of Economic Co-operation Development). (1998). Test guidelines No. 408: Repeated dose 90-day oral toxicity study in Rodents. OECD Publishing, Paris.

5. Féres, C. A., Madalosso, R. C., Rocha, O. A., Leite, J. P., Guimarães, T. M., Toledo, V. P., &Tagliati, C. A. (2006). Acute and chronic toxicological studies of *Dimorphandramollis* in experimental animals. Journal of ethnopharmacology,108(3), 450–456. https://doi.org/10.1016/j.jep.2006.06.002.

6. Teo, S.K., Stirling, D.I., Thomas, S.D., Hoberman, A.M., Kiorpes, A.L., &Khetani, V.D. (2002). A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. Toxicology, 179 (3), 183-196.

7. Gad, S. (2008). Repeat Dose Toxicity Studies. In: Preclinical Development Handbook: John Wiley & Sons, p. 213-232.

8. Speakman, J. R.&McQueenie, J. (1996). Limits to Sustained Metabolic Rate: The Link between Food Intake, Basal Metabolic Rate, and Morphology in Reproducing Mice, *Musmusculus*. Physiological Zoology, 69(4), 746–769.

9. Shokryazdan, P., FaselehJahromi, M., Liang, J. B., Kalavathy, R., Sieo, C. C., &Ho, Y. W. (2016). Safety Assessment of Two New Lactobacillus Strains as Probiotic for Human Using a Rat Model. PloS one, 11(7), e0159851. https://doi.org/10.1371/journal.pone.0159851.

10. Mukinda, J. T., &Syce, J. A. (2007). Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. Journal of ethnopharmacology, 112(1), 138–144. https://doi.org/10.1016/j.jep.2007.02.011.

11. Gribaldo L. (2002). Haematotoxicology: scientific basis and regulatory aspects. Alternatives to laboratory animals:ATLA, 30(2), 111–113. https://doi.org/10.1177/026119290203002S1.

12. Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Van Deun, K., Smith, P., Berger, B., & Heller, A. (2000). Concordance of the toxicity of

pharmaceuticals in humans and in animals. Regulatory toxicology and pharmacology, 32(1), 56–67. https://doi.org/10.1006/rtph.2000.1399.

13. Wolf, P. L. (1999). Biochemical diagnosis of liver disease. Indian journal of clinical biochemistry, 14(1), 59–90. https://doi.org/10.1007/BF02869152.

14. Devarbhavi, H. (2012). An Update on Drug-induced Liver Injury. Journal of clinical and experimental hepatology, 2(3), 247–259. https://doi.org/10.1016/j.jceh.2012.05.002.

15. EMEA. (2008). Non-Clinical Guideline on Drug-InducedHepatotoxicity. European Medicines Agency.

16. Arneson, W. &Brickell, J. (2007) Assessment of Liver Function. In: Arneson W, Brickell J, editors.Clinical Chemistry: A Laboratory Perspective, 1st ed. Philadelphia: F.A. Davis Company, 233-266.

17. Aniagu, S. O., Nwinyi, F. C., Olanubi, B., Akumka, D. D., Ajoku, G. A., Izebe, K. S., Agala, P., Agbani, E. O., Enwerem, N. M., Iheagwara, C., &Gamaniel, K. S. (2004). Is *Berlinagrandiflora* (Leguminosae) toxic in rats?. Phytomedicine: International Journal OfPhytotherapy and Phytopharmacology, 11(4), 352–360. https://doi.org/10.1078/0944711041495155.

18. Schreiner, G.E. & Maher, J.F. (1965). Toxic Nephropathy.The American Journal of Medicine, 38, 409-449.

19. Greaves, P. (1990). Histopathology of preclinical toxicity studies. In: Interpretation and relevance in drug safety evaluation. Academic press, Elsevier, Amsterdam, New York, Oxford,pp - 824.