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Acute and 28-day repeated oral toxicological evaluation of Kuruthi Azhal Chooranam – a Siddha preparation on rodents

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ABSTRACT

Objective: To determine the effect of phytochemicals in acute and repeated dose of 28-day oral toxicity of Kuruthi Azhal Chooranam (KAC) in Sprague Dawley rats of both sexes.
Methods: Acute oral toxicity was conducted with 2 000 mg/kg body weight of KAC orally and the treated animals were observed for signs of toxicity at 30 min, 1, 2, 4 and 24 h and for up to 14 days. In repeated 28-day oral toxicity study, the KAC formulation was administered orally with 600, 900 and 1 200 mg/kg body weight/day to all the three groups of rats. The animals were observed for clinical signs of toxicity, mortality and morbidity throughout the study. Also body weight, feed consumption, haematological, plasma biochemistry and serum electrolytes, gross pathology, weights of the organ and histology were studied for no-observed-adverse-effect level. High dose of KAC formulation and control reversal groups were also included for delayed toxic effects determination.
Results: In the acute toxicity study of KAC formulation, 2 000 mg/kg body weight dose exhibited no toxic signs and mortality during study. In sub-acute 28-day repeated dose toxicity study, there was no significant difference found between control and KAC treated groups (body weight, haematology, biochemistry and serum electrolytes). No abnormalities was found in gross pathology, organs weight and histological observation after KAC treatment.
Conclusions: The current study suggests that LD_{50} of KAC was > 2 000 mg/kg and no-observed-adverse-effect level was > 1 200 mg/kg/day in rats. KAC could be used as Siddha drug for various indications.

1. Introduction

Herbal medicine plays an important role in the healthcare of many developing countries because of distinct benefits such as the presence of diverse bioactive compounds that can act synergistically to treat contagious diseases, even though it does not reach substantial level at developed nations due to the lack of safety and efficacy profiles. The herbal medicine has been a step of universal developments in knowledge, innovations and current practices. Siddha is a traditional medical system, practiced by South India and is increasingly recognized as an alternate approach. Promising studies have been conducted in recent days to know the efficacy of Siddha preparations in the practices of cardiometabolic diseases, skin diseases, arthritis, gastrointestinal and dermatological diseases[1-3]. Siddha medicine believes that formulation process is more important for its efficacy whereas allopathic believes in the chemical constituents of the efficacy. *Cuminum cyminum* (*C. cyminum*) has been practiced as traditional medicine in South India for the treatment of hypertension.

Kuruthi Azhal Chooranam (KAC) is a unique formulation

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The study protocal was followed according to Guide for the Care and Use of Laboratory Animals and approved by Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India.

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consisting of *C. cyminum* as the major ingredient and 11 other active constituents such as *Zingiber officinale*, *Allium cepa, Cyamopsis tetragonoloba, Emblica officinalis, Citrus medica, Coriandrum sativum, Leucas aspera, Melothria perpusilla, Solanum trilobatum, Azadirachta indica* and *Saccharum officinarum*. These herbs have numerous pharmacological properties like antioxidant and anticancer activities, *etc.*[4-9].

Several therapeutic effects would be achieved with combinations of natural drugs, due to their multi pharmacological actions and absence of toxicity with the safety assessment. Hence, this study was designed to determine the acute and sub-acute oral toxicity of KAC formulation.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid and quercetin were purchased from Sigma Chemicals, United States of America. Diagnostic and biochemical kits were purchased from Accurex Biomedical Pvt. Ltd., (Mumbai, India).

2.2. Preparation of formulation and authentication

KAC prepared using 12 ingredients of *C. cyminum, Zingiber officinale, Allium cepa, Cyamopsis tetragonoloba, Emblica officinalis, Citrus medica* and leaves of *Coriandrum sativum* were procured from organic super market at Kottivakkam, Chennai. Leaves of *Leucas aspera, Melothria perpusilla, Solanum trilobatum, Azadirachta indica* bark and *Saccharum officinarum* were collected from Cheiyar area, Kanchipuram Distrrict of Tamil Nadu, India. All the individual herbs were authenticated at Siddha Central Research Institute by Dr. Sasikala Ethirajulu, Assistant Director/ Pharmacognosist. The final formulation was lyophilized using Christ Alpha 1-2 LD Plus, USA and stored in vacuum desiccators until used.

2.3. Animals and husbandry

Sprague-Dawley rats of both sexes weighing 130–160 g were obtained from Central Animal Facility, Sri Ramachandra University, Chennai, India. In acute oral toxicity study, animals were housed individually in polypropylene cages in environmental condition at a temperature of (22 ± 2) ℃ with 40%–65% relative humidity and photoperiod of 12 h (light: dark). They were provided with pelleted feed (Nutrilab Rodent, Tetragon Chemie Pvt Ltd., Bangalore, India) and purified water *ad libitum*. All the animals were housed 7 days in the laboratory conditions. The study protocal was followed according to Guide for the Care and Use of Laboratory Animals and approved by Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India (IAEC/XIX/SRU/132/2010).

2.4. Phytochemical screening and quantification of secondary metabolites

Lyophilized KAC was subjected to preliminary phytochemicals analysis which was performed using standard protocols[10]. Total antioxidant and reducing capacity were evaluated[11,12].

2.5. Acute oral toxicity study

Acute oral toxicity was conducted with young adult Sprague –Dawley (SD) female (non-pregnant and nulliparous) rats with body weight of about 120–130 g in the study. Control group was treated with 0.5% carboxy methyl cellulose (CMC) and treatment group was administered with KAC of 2 000 mg/kg body weight prepared in 0.5% CMC. Immediately after administration, clinical signs of toxicity, mortality and morbidity were recorded at 30 min, 1, 2, 4 h and for up to 14 days of experimental period. Animal body weights were recorded prior to dosing (day 0), weekly once and at the end of experimental period (day 14) animals were subjected to gross pathology and organs were examined for pathological observation[13].

2.6. The 28-day repeated oral dose toxicity study

The repeated 28-day oral dose toxicity study was conducted with young Sprague–Dawley rats of either sex weighing about 120–140 g. Rats were randomized into six groups in each (5 males and 5 females = 10). Group I was considered as vehicle control treated with 0.5% CMC, and the treatment Group II–IV were dosed with KAC formulation at the concentrations of 600, 900 and 1 200 mg/kg body weight respectively, and daily for 28 days. The satellite vehicle control and 1 200 mg/kg body weight was used to determine delayed/ persistence toxicity and recovery from effect. The satellite Group V–VI were observed for reversibility of toxicity effects for 14 days without the administration of dose and necropsied at the end of 42 days. Toxic symptoms, signs of toxicity and mortality were observed daily. Body weight, feed and water intake were monitored once in a week. All the rats were individually observed for morbidity and mortality after dose administration. Rats were fasted overnight prior to blood samples collection. For haematological and biochemical parameters the blood samples were collected from retro orbital sinus with and without anticoagulant[14,15].

Haematological parameters like white blood cells, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet were analysed by haematology analyzer (PE 6000). The cold centrifuged and separated plasma was used to estimate the glucose, triglyceride, cholesterol, creatinine, urea and total protein with standard diagnostic kits using semi-automatic

biochemical analyser (Star-21 Plus, India). The following enzymes levels such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and other constituents were also estimated in plasma.

2.7. Histopathology examinations

Necropsy was done at the end of the treatment day and the satellite group was euthanized and pathology was performed at the end of the reversibility period (42nd day). Immediately after blood collection, rats were euthanized with isoflurane and gross pathology observations of vital organs like brain, liver, heart, spleen and kidneys were examined. Gross lesions and respective organ weight were recorded. The animal tissues of control and high dose group were fixed in 10% neutral buffered formalin and sections of 5–6 mm, stained with haematoxylin and eosin and examined under a light microscope (Olympus CHO2).

2.8. Statistical analysis

Data were presented as mean ± SE and subjected to statistical analysis by student's *t*-test ($P \leqslant 0.05$). GraphPad Prism 4.0 was used to determine significant difference between the control and treatment.

3. Results

3.1. Preliminary screening and quantitative determination of phytochemicals and vitamins

Despite the usage of the plants in folklore medicine over ages, recently pharmacology and toxicology of plants begun to receive attention. The preliminary phytochemical analysis showed the presence of phenol, tannins, saponin, glycosides, alkaloids, quinones, flavones and anthroquinones in KAC formulation. KAC contained higher amount of phenol [(18.56 ± 1.16) mg/g extract], tannin [(1.46 ± 0.03) mg/g extract] and flavonoid [(5.47 ± 0.47) mg/g extract]. Reducing capacity was found in higher amounts [(43.12 ± 3.23) mg extract equivalent/100 μg of vitamin C] when compared to total antioxidant [(10.12 ± 1.31) mg extract equivalent/100 μg of vitamin E].

3.2. Acute toxicity study

Acute toxicity of KAC was studied at 2000 mg/kg body weight, and there was no mortality and any other treatment related signs of toxicity in duration of study. The KAC did not show any significant change in body weight of animals when compared with control animals (Table 1). The gross pathological examination showed no toxic effects in the internal organs of the treated animals. This study demonstrated a lack of toxicity after the oral administration of KAC at a dose of 2000 mg/kg body weight in the acute toxicity.

3.3. The 28-day repeated dose toxicity study

In 28-day repeated dose toxicity study, animals were treated orally at 600, 900 and 1200 mg/kg body weight of KAC formulations. Neither clinical signs of toxicity, morbidity nor mortality was recorded in KAC formulation treated groups throughout the study period. Body weight gain was found high in the low-dose (600 mg/kg body weight) treated animals but there was no significant difference between the control and the low dose treatment. The other group was compared with the vehicle treated animals (Tables 2 and 3). The results of feed consumption of control and KAC treated rats are shown in Table 3. There was no significant difference in feed consumption between the vehicle and KAC treated animals throughout the study (Table 3).

3.4. Determinations of haematological, serum electrolytes, biochemical parameters and relative organ weights

There were no significant differences in any of the tested haematological parameters which were observed between the

Table 1
Acute oral toxicity effect of KAC on Sprague–Dawley rats.

Group	Treatment	Body weight (g)				
		0th day	1st day	2nd day	7th day	14th day
I	Control	126.00 ± 1.15	118.67 ± 0.67	121.90 ± 4.00	119.67 ± 8.69	136.00 ± 7.37
II	KAC (2000 mg/kg body weight)	126.33 ± 2.85	119.33 ± 1.45	119.00 ± 3.06	132.00 ± 2.08	147.00 ± 11.24

Table 2
Effect of KAC on body weight of Sprague–Dawley rats. g.

Group	Treatment (mg/kg body weight/day)	1st week	2nd week	3rd week	4th week	5th week	6th week
I	Control	127.80 ± 5.08	131.16 ± 7.20	151.88 ± 10.69	164.12 ± 9.71		
II	600	134.20 ± 2.44	153.46 ± 9.49	180.26 ± 3.79	197.58 ± 11.06		
III	900	133.80 ± 8.17	137.62 ± 10.67	154.00 ± 13.41	170.46 ± 7.78		
IV	1200	135.90 ± 3.34	147.72 ± 7.20	166.92 ± 3.78	179.20 ± 6.07		
V (satellite)	Control	131.60 ± 2.33	134.04 ± 9.49	156.92 ± 0.30	176.68 ± 0.14	189.60 ± 4.98	179.20 ± 6.07
VI (satellite)	1200	133.50 ± 6.58	140.80 ± 11.16	156.59 ± 11.63	179.36 ± 15.16	192.66 ± 14.26	208.24 ± 10.86

Values are expressed in mean ± SEM; $n = 10$ (5/sex); Statistical analysis was performed using student's *t*-test using GraphPad Prism 4.0.

Table 3

Effect of KAC on feed weight of Sprague–Dawley rats. g.

Group	Treatment (mg/kg body weight/day)	1st week	2nd week	3rd week	4th week	5th week	6th week
I	Control	60.12 ± 6.30	65.72 ± 5.83	89.62 ± 0.93	90.94 ± 3.13		
II	600	68.86 ± 5.40	72.32 ± 3.35	92.92 ± 2.33	103.52 ± 6.14		
III	900	56.12 ± 7.00	63.82 ± 0.75	89.32 ± 3.89	92.75 ± 9.03		
IV	1 200	72.04 ± 2.29	66.38 ± 5.96	95.78 ± 1.62	95.02 ± 0.92		
V (satellite)	Control	67.28 ± 9.49	60.93 ± 6.17	79.64 ± 6.81	92.93 ± 2.46	89.26 ± 3.79	90.62 ± 5.73
VI (satellite)	1 200	64.64 ± 4.30	74.30 ± 6.30	76.66 ± 4.30	92.76 ± 2.67	91.63 ± 4.71	90.85 ± 6.21

Values are expressed in mean ± SEM; n = 10 (5/sex); Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

vehicle and the KAC formulations treated groups, and results were summarised in Table 4. KAC formulations did not produce any significant changes in the plasma biochemical parameters such as glucose, cholesterol, triglyceride, bilirubin and liver and kidney marker parameters like ALT, ALP, GGT, albumin, total protein, urea and creatinine at any of the treated dose levels when compared to the vehicle treated rats (Table 5). There were no significant changes found in the serum Na, K, Ca, Cl, and pH between vehicle and KAC treated animals (Table 6). Administration of KAC formulations had no treatment related changes in organ and relative organ weight of control and high dose treated animals (Tables 7 and 8). Haematological profile of experimental animals showed no significant difference between the control and treatment group of the both sexes. These results indicated that KAC did not affect haematopoiesis and the formation of blood cellular components.

Table 4

Effect of KAC on haematological profile of Sprague-Dawley rat.

Group	Treatment (mg/kg body weight/day)	WBC (10^3/μL)	RBC (10^6/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/μL)
I	Control	7.08 ± 1.65	2.89 ± 0.10	9.77 ± 0.79	11.76 ± 0.27	40.37 ± 0.25	72.86 ± 5.48	152.45 ± 3.68	385.80 ± 9.44
II	600	7.95 ± 0.31	3.90 ± 0.57	14.10 ± 0.72	17.39 ± 2.36	45.05 ± 0.29	54.54 ± 5.16	80.02 ± 6.00	261.60 ± 4.26
III	900	6.10 ± 0.29	4.67 ± 1.13	14.69 ± 0.05	24.65 ± 2.44	44.81 ± 0.25	30.68 ± 2.39	68.84 ± 1.00	267.00 ± 8.83
IV	1 200	6.72 ± 0.43	4.46 ± 0.85	12.53 ± 2.96	19.71 ± 4.22	44.54 ± 0.23	28.39 ± 0.77	64.13 ± 0.01	265.30 ± 0.36
V (satellite)	Control	7.19 ± 2.13	3.08 ± 0.65	14.28 ± 0.01	12.28 ± 2.77	38.38 ± 0.04	26.97 ± 4.41	53.67 ± 2.82	441.98 ± 0.41
VI (satellite)	1 200	12.40 ± 1.16	5.99 ± 0.45	14.44 ± 0.19	31.67 ± 7.23	50.63 ± 0.63	30.66 ± 1.12	61.26 ± 5.29	363.21 ± 8.62

Values are expressed in mean ± SEM; n = 10 (5/sex); Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

Table 5

Effect of KAC on plasma biochemical parameters of Sprague–Dawley rat.

Biochemical parameters	Treatment (mg/kg body weight/day)					
	Control	600	900	1 200	Control (satellite)	1 200 (satellite)
Glucose (mg/dL)	86.14 ± 0.87	90.00 ± 5.91	85.23 ± 3.88	81.67 ± 4.09	92.26 ± 1.38	89.10 ± 1.36
Cholesterol (mg/dL)	75.59 ± 1.02	62.85 ± 4.72	75.87 ± 3.36	86.84 ± 6.59	69.25 ± 7.25	64.04 ± 8.61
Triglyceride (mg/dL)	48.05 ± 3.16	49.66 ± 3.97	46.95 ± 0.85	45.38 ± 7.20	79.70 ± 4.19	81.27 ± 0.82
Total protein (g/dL)	6.09 ± 0.12	6.10 ± 0.19	6.06 ± 0.01	5.92 ± 0.02	5.71 ± 0.09	6.08 ± 0.01
Urea (mg/dL)	41.53 ± 2.28	37.71 ± 0.13	40.64 ± 4.01	35.37 ± 0.19	43.71 ± 0.48	44.08 ± 0.74
Albumin (gm/dL)	1.71 ± 0.10	2.71 ± 0.03	2.50 ± 0.19	2.74 ± 0.04	1.34 ± 0.14	1.31 ± 0.06
Creatinine (mg/dL)	0.57 ± 0.00	0.49 ± 0.01	0.40 ± 0.02	0.43 ± 0.05	0.83 ± 0.01	0.75 ± 0.00
Total bilirubin (mg/dL)	0.97 ± 0.01	0.62 ± 0.03	0.61 ± 0.10	0.76 ± 0.01	0.94 ± 0.11	1.04 ± 0.02
ALT (IU/L)	35.75 ± 0.27	34.07 ± 4.87	31.15 ± 2.14	43.77 ± 1.30	59.28 ± 0.09	56.58 ± 0.53
ALP (IU/L)	378.78 ± 35.59	394.9 ± 33.06	381.6 ± 12.70	378.9 ± 20.00	454.20 ± 24.11	529.01 ± 24.62
GGT (IU/L)	6.05 ± 0.27	4.26 ± 0.74	4.27 ± 0.29	4.35 ± 0.10	4.76 ± 0.67	3.84 ± 0.22

Values are expressed in mean ± SEM; n = 10 (5/sex); Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

Table 6

Effect of KAC on serum electrolyte in Sprague–Dawley rats. mmol/L.

Group	Treatment (mg/kg body weight/day)	Total calcium	Potassium	Sodium	Chloride	pH
I	Control	2.72 ± 0.09	4.41 ± 0.15	154.29 ± 1.13	98.14 ± 1.15	7.72 ± 0.01
II	600	3.66 ± 0.03	4.68 ± 0.10	148.32 ± 2.79	102.89 ± 4.69	7.77 ± 0.02
III	900	2.69 ± 0.08	3.95 ± 0.09	151.72 ± 2.98	104.89 ± 3.42	7.46 ± 0.02
IV	1 200	2.52 ± 0.03	4.83 ± 0.13	147.07 ± 3.86	93.89 ± 2.44	7.49 ± 0.01
V (satellite)	Control	2.29 ± 0.05	4.27 ± 0.12	149.67 ± 4.82	108.25 ± 4.63	7.61 ± 0.02
VI (satellite)	1 200	3.71 ± 0.11	3.18 ± 0.22	142.98 ± 3.71	99.53 ± 3.67	7.28 ± 0.01

n = 10 (5/sex); values are expressed in mean ± SEM; Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

Table 7

Effect of KAC on organ weight of Sprague-Dawley rats. g.

Group	Treatment (mg/kg body weight/day)	Brain	Heart	Liver	Kidney	Spleen	Testes	Ovaries	Adrenals
I	Control	1.82 ± 0.03	0.76 ± 0.04	7.76 ± 0.62	1.65 ± 0.11	0.87 ± 0.05	2.56 ± 0.29	0.19 ± 0.02	0.05 ± 0.01
II	600	1.75 ± 0.01	0.90 ± 0.07	6.87 ± 0.18	1.50 ± 0.01	0.76 ± 0.05	2.34 ± 0.40	0.27 ± 0.24	0.06 ± 0.01
III	900	1.83 ± 0.04	0.86 ± 0.07	8.11 ± 0.19	1.75 ± 0.08	0.91 ± 0.07	2.89 ± 0.24	0.11 ± 0.02	0.06 ± 0.01
IV	1 200	1.90 ± 0.02	0.78 ± 0.02	7.44 ± 0.14	1.67 ± 0.05	0.94 ± 0.05	2.39 ± 0.17	0.13 ± 0.01	0.07 ± 0.01
V (satellite)	Control	1.57 ± 0.01	0.86 ± 0.02	7.50 ± 0.21	1.52 ± 0.01	0.86 ± 0.03	2.80 ± 0.08	0.22 ± 0.02	0.05 ± 0.05
VI (satellite)	1 200	1.59 ± 0.01	0.93 ± 0.01	6.49 ± 0.05	1.57 ± 0.02	0.89 ± 0.02	2.91 ± 0.37	0.21 ± 0.08	0.06 ± 0.01

Values are expressed in mean ± SEM; n = 10 (5/sex); Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

Table 8

Effect of KAC on relative organ weight of Sprague-Dawley rats. g.

Group	Treatment (mg/kg body weight/day)	Brain	Heart	Liver	Kidney	Spleen	Testes	Ovaries	Adrenals
I	Control	1.12 ± 0.04	0.46 ± 0.02	4.71 ± 0.30	1.00 ± 0.05	0.54 ± 0.03	1.47 ± 0.15	0.06 ± 0.01	0.03 ± 0.01
II	600	0.98 ± 0.08	0.50 ± 0.08	3.77 ± 0.32	0.84 ± 0.07	0.41 ± 0.04	1.11 ± 0.20	0.08 ± 0.01	0.03 ± 0.01
III	900	1. 12 ± 0.07	0.51 ± 0.03	4.88 ± 0.33	1.05 ± 0.06	0.55 ± 0.06	1.68 ± 0.19	0.07 ± 0.01	0.03 ± 0.01
IV	1 200	1.08 ± 0.06	0.45 ± 0.03	4.20 ± 0.26	0.95 ± 0.06	0.53 ± 0.04	1.42 ± 0.09	0.07 ± 0.01	0.04 ± 0.01
V (satellite)	Control	1.16 ± 0.01	0.52 ± 0.01	3.82 ± 0.04	0.89 ± 0.01	0.52 ± 0.01	1.53 ± 0.01	0.06 ± 0.03	0.04 ± 0.01
VI (satellite)	1 200	0.79 ± 0.01	0.46 ± 0.01	4.26 ± 0.05	0.77 ± 0.02	0.43 ± 0.01	0.88 ± 0.23	0.11 ± 0.04	0.03 ± 0.01

Values are expressed in mean ± SEM; n = 10 (5/sex); Statistical analysis was performed using student's t-test using GraphPad Prism 4.0.

3.5. Histopathology

Macroscopic, gross pathological and histopathological observation of the organs and tissues did not exhibit any abnormal changes of control and treatments. Histopathological results of brain, heart, liver, kidney, stomach and spleen of control, high dose KAC treated and satellite groups are shown in Figures 1 and 2. Histopathological evaluation of satellite control and high dose of KAC treated animals showed no abnormalities in the architecture of organs.

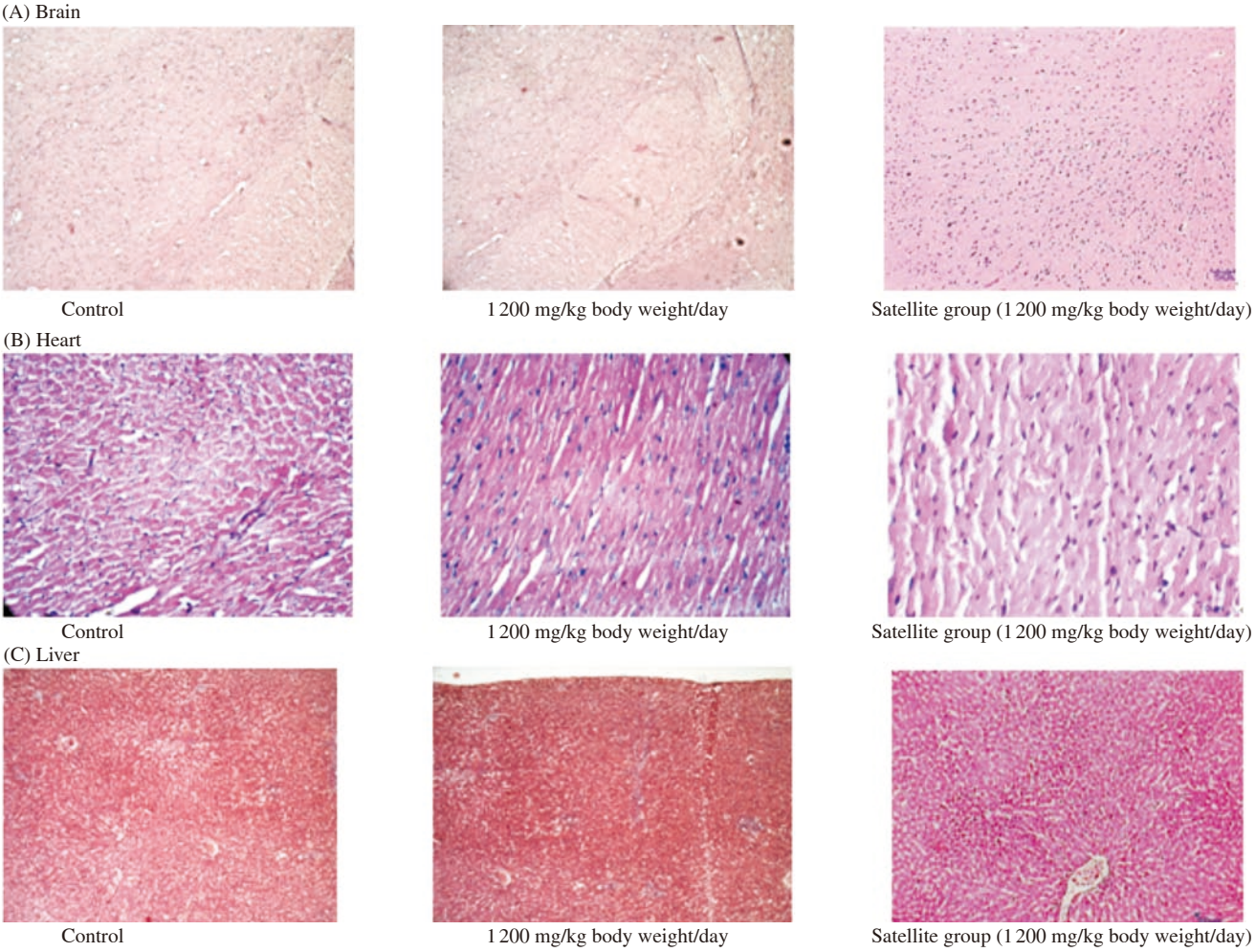

Figure 1. Effect of KAC on histopathologic changes of brain, heat and liver of Sparague–Dawley rats.

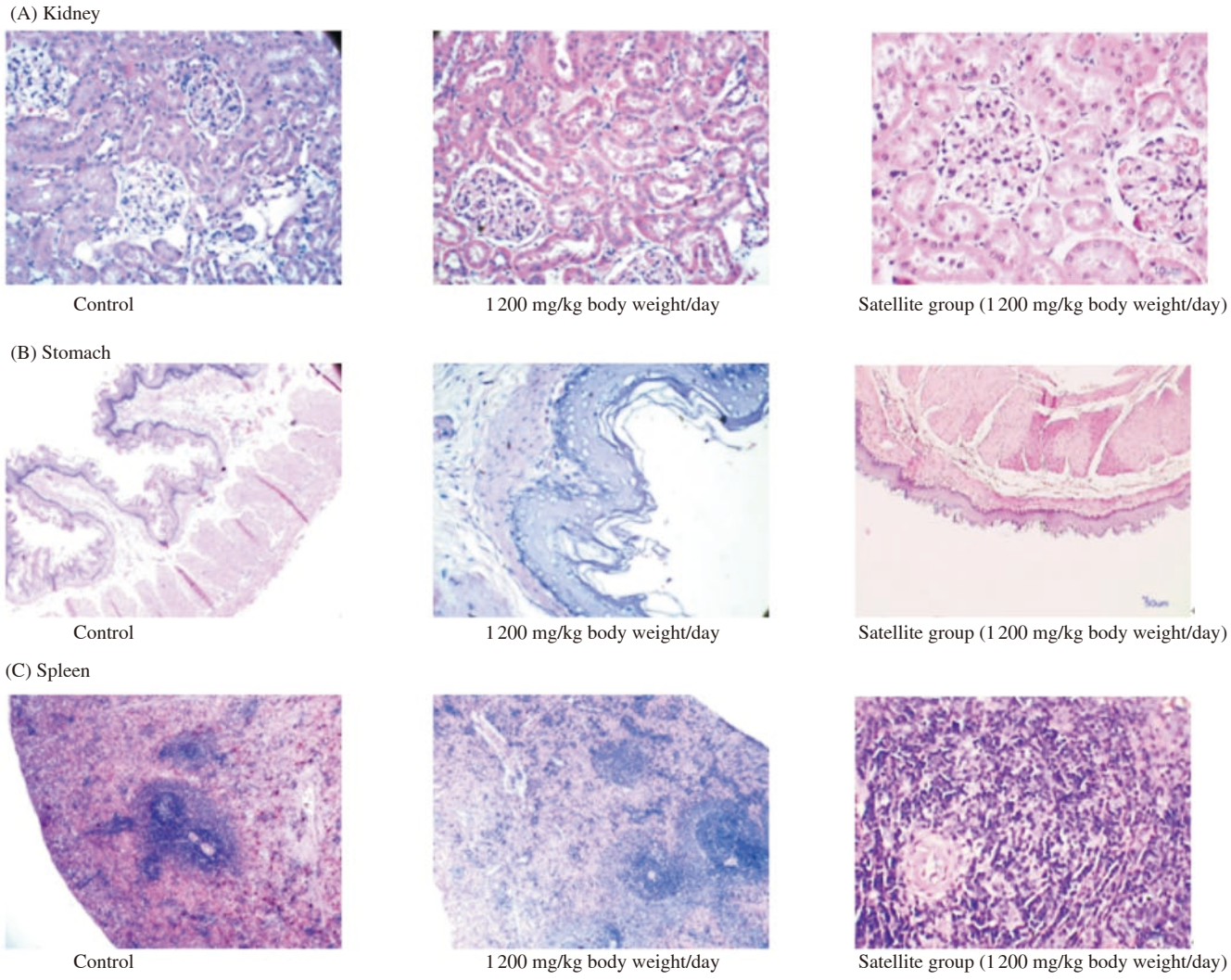

Figure 2. Effect of KAC on histopathologic changes of kidney, stomach and spleen of Sparague–Dawley rats.

4. Discussion

The preliminary phytochemical analysis showed presence of phenol, tannins, saponin, glycosides, alkaloids, quinones, flavones and anthroquinones in KAC formulation. This study coincides with the finding of Mohammadi *et al.*[16] who reported that phenol, triterpenoids and flavonoids have a wide spectrum of pharmacological effects such as antioxidant activity. Alkaloids were showed with anti-inflammatory effects[17]. Non-clinical studies of poly herbal formulations provide scientific justification of their traditional use, safety and efficacy.

Acute toxicity of KAC was studied at 2 000 mg/kg body weight and there was no mortality and any other treatment related signs of toxicity in duration of study. Similar result was also observed by Nanthini *et al.*[18] who stated that at 2 000 mg/kg body weight of *Mandoora chooranam* did not showed mortality, signs of toxicity and any behavioral effects.

In the 28-day repeated dose toxicity study, animals were treated orally at 600, 900 and 1 200 mg/kg, body weight of KAC formulations. There were no significant differences in feed consumption between the vehicle and KAC treated animals throughout the study. Similarly, Kadukkai maathirai and Velvanga parpam treated rats did not show any significant changes of feed consumption and body weight during the experimental period of 28 days[19,20].

There were no significant differences in any of the tested haematological parameters that were observed between the vehicle and the KAC formulations treated groups. Similarly, haematopoietic system serves as an important target for toxic chemicals and a sensitive marker by Chandiran *et al.*[21] for the evaluation of toxicity. A Siddha formulation Velvanga parpam did not affect the biochemical and haematological changes of treated rats[20] and also a Siddha medicine, Nuna kadugu treated animals did not exhibit any significant changes in biochemical and heamtological parameters[22].

Histopathological evaluation of satellite control and high dose of KAC treated animals showed no abnormalities in the architecture of organs. The present study coincides with earlier findings of Kumar and Kumar[23] who reported that *Cycas circinalis* and *Ionidium suffruticosum* did not show any histopathological effects in stomach, lung, spleen, kidney, live and brain of the rats. Siddha drug Lagu Seena Chooranam treatment did show any histopathological changes of kidney, liver, heart, lungs, spleen, pancreas, brain and ovaries of the rats[24]. The present study showed that there were no significant

differences between the control and KAC treated rats which were evidenced by the histopathological evaluation of kidney which had normal architecture. Similarly, Sudha *et al.*[20] who reported that Siddha drug Velvanga parpam did not show any histopathological changes of treated rat when compared to control.

The present investigation revealed that 28-day repeated oral dose with KAC exhibited no treatment related toxicity signs, gross pathology and histopathological abnormalities in rats at the tested dose levels. The data suggest that accumulation of any potential active constituents resulting from KAC consumption does not lead to toxicity. The treated doses of KAC in rats were greater than any doses anticipated for human consumption. The treatment of KAC did not show any acute and repeated dose toxicity effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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