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Acute and sub-chronic toxicity evaluation of a standardized green coffee bean extract (CGA-7™) in Wistar albino rats

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Abstract

Objective: Despite having numerous physiological benefits, toxicological assessment of green coffee beans is sparce. Here, we document the oral acute and sub-chronic toxicity of a standardized decaffeinated green coffee bean extract containing 50% chlorogenic acids (CGA-7™) in rats.

Methods: We have performed a limit test at single oral dose of 2000 mg/kg to evaluate the acute toxicity in female Wistar rats. Furthermore, repeated dose 90-day toxicity study was conducted to assess the risk of long-term use of CGA-7.

Result: A 14-day observation revealed no clinical signs of toxicity or mortality in animals at 2000 mg/kg acute oral dose of CGA-7. The administration of 250, 500, and 1000 mg/kg CGA-7 showed significant alterations in some parameters such as food consumption, relative organ weights of brain and spleen, haematological and biochemical parameters compared to control. These changes were not consistent and dose-dependent throughout the study. Furthermore, the changes were within the physiological range and toxicologically insignificant. CGA-7 did not affect the normal metabolism and physiology of the animals up to 1000 mg/kg dose. Macroscopic and histological examination of organs did not reveal any organ toxicity.

Conclusion: Finally, the findings from this study suggest the safety of green coffee bean extract.

Keywords

Coffee beans, chlorogenic acids, safety, rats, physiology

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Introduction

Alternative and complementary medicine has significantly influenced the healthcare management across the globe.[1] Plant-based natural products are used as dietary supplements, functional ingredients in food and beverages, and therapeutic interventions. Due to their large-scale usage, it is necessary to validate the safety alongside the efficacy of these herbal products.[2] In general practice, herbs, as in use documented in the traditional systems of medicine, have been considered to have low toxicity.[3] However, it is important to build the safety profile of the herbals based on thorough scientific studies to overrule the plausible toxic effects if consumed.

Green coffee beans are documented to possess various healthcare benefits such as antidiabetic, antioxidant, antihypertensive, and anti-obesity effects.[4–10] The physiological attributes of green coffee beans are majorly due to the presence of chlorogenic acids (CGAs).[11,12] However, there are a few reports on the adverse effects of CGA. Previously, Fan et al. studied the effect of hydroxycinnamic acids including CGA, causing DNA damage in the presence of Cu(II) ions.[13] Du et al.[14] reported that a high dose of CGA administered to male Wistar rats could induce inflammation reaction, as evident from the increased leukocyte count, cytokine levels, and reduced antioxidant status. In another study, CGA was shown to have prooxidant effect leading to the induction of apoptosis in myeloid leukaemia cells via enhanced intracellular reactive oxygen species (ROS) generation.[15] Here, we have investigated the in vivo toxicity of a standardized green coffee bean extract containing 50% CGA. The safety of the extract was established based on acute and sub-chronic toxicity evaluations.

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Materials and methods

Plant extract

CGA-7 is a standardized decaffeinated green coffee bean extract from *Coffea arabica*. The CGA content of the extract was quantified to be not less than 50% by high-performance liquid chromatography (HPLC) method (Supplementary File 1). CGA-7 contains seven isomers of CGA, 5-caffeoylquinic acid being the major isomer present (>25%). The CGA-7 extract in powder form was obtained from the Department of Quality Control, Vidya Herbs Pvt Ltd, Bangalore, India. The extract was freshly dissolved in 0.9% physiological saline for daily oral administration.

Animals

Wistar albino rats (male and female) were used for the toxicity studies. The animals were purchased from Biogen Pvt Ltd, Bangalore, India. All the animals were housed in animal rooms with temperature range of 22 ± 3°C, relative humidity 40%–70% and lighting of 12 h per day. The animals were kept in polypropylene cages (length × width × height: 300 × 400 × 210 mm^3), five rats per cage. The rats were fed with commercially available pellet diet and water ad libitum. The experiments were performed after the approval by the Institutional Animal Ethics Committee (IAEC; VHPL/PCL/IAEC/02/13). The sample size for the toxicity studies was decided in accordance with the Organisation of Economic Co-operation Development (OECD) guidelines.

Chemicals

Kits for measuring the total bilirubin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL-c), urea, total protein, creatinine, alkaline phosphatase (ALP), glucose, calcium, and phosphorus were procured from Robonik India Pvt Ltd.

Acute oral toxicity study

The single-dose acute oral toxicity test was performed in accordance with OECD guideline No. 425.[16] Five nulliparous and non-pregnant female Wistar rats aged 10–12 weeks were housed in a polypropylene cage. The rats were acclimatized for 7 days before the experiment. A limit test was performed where a single animal received a single dose of 2000 mg/kg CGA-7 dissolved in 0.9% saline solution and observed for 24 h. Following the observation for mortality, the remaining animals were given the limit dose. After dosing, the animals were observed individually once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter for a total of 14 days.

Observations included mortality and clinical signs such as changes in skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity, and behaviour pattern. Attentions were given to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma. The animal body weights were recorded shortly before the administration of CGA-7 and at days 7 and 14 post-administration. The animals were euthanized at the end of study, by intraperitoneal injection of ketamine/xylazine (80/10 mg/kg). The vital organs were macroscopically examined for toxicity.

Sub-chronic toxicity study

The repeated dose 90-day sub-chronic toxicity study was performed in compliance with the OECD Guideline (408) for the testing of chemicals with modifications.[17] A total of 100 (50 male and 50 female) healthy, Wistar rats (6–8-week-old) weighing 160–200 g were used for sub-chronic toxicity evaluation. The animals of both sexes were allocated to five groups ($n = 20$; 10 males and 10 females). Group 1 animals served as vehicle control group and Group 2–4 rats were administered orally with 250, 500, and 1000 mg/kg doses of CGA-7, respectively. Group 5 was the test reversal group orally administered with 1000 mg/kg CGA-7 for 90 days with a follow-up period of 14 days additionally without treatment.

All the animals were observed daily for the behavioural pattern, clinical signs, and mortality till the end of study. The body weight, feed, and water intake were recorded daily. At the end of study, the rats were euthanized by an overdose of anaesthesia (ketamine (80 mg/kg)/xylazine (10 mg/kg) intraperitoneal). Blood samples were collected by cardiac puncture; serum and plasma samples were used for biochemical (Automatic Chemistry Analyzer; URIT-8021A, Unitron Biomedicals) and haematological assessments (Automatic Haematology Analyzer; F-19E, Unitron Bio Medicals, Bangalore, India), respectively. The organs were carefully excised, weighed, and observed macroscopically. The relative organ weights were determined.

Histopathology

After necropsy, parts of liver, heart, brain, spleen, kidneys, testes, and ovaries from the control and extract-treated groups were collected for histological studies. The organ samples were washed in saline and stored in 10% formalin for histopathological examination.

Statistical analysis

The data were statistically analysed by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test using

Table 1. Animal body weight gain during acute oral toxicity of CGA-7 in female Wistar rats.

Dose (mg/kg body weight)	Body weight (g)	Body weight (g)		% Change in body weight	
	Day 0	Day 7	Day 14	Days0–7	Days 0–14
2000	160.07 ± 3.97	173.20 ± 9.08	180.23 ± 10.45	8.16 ± 3.20	12.55 ± 4.12

CGA: chlorogenic acid.
Values are mean ± standard deviation (SD) ($n = 5$).

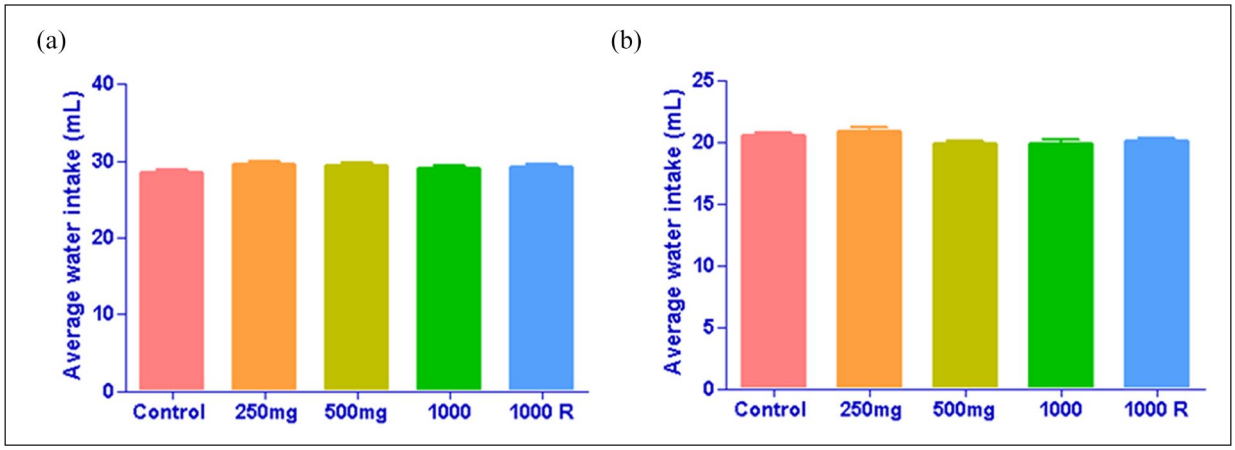

Figure 1. Effect of CGA-7 extract treatment on average water consumption of (a) male and (b) female rats.
Values are expressed as mean ± SEM ($n = 10$ for each group).
*p value of <0.05 was considered significant using one-way ANOVA followed by Dunnet's t test. * denotes significant difference compared to control.

GraphPad Prism version 5.0. The values are expressed as mean ± SEM.

Results

Acute oral toxicity

No mortality and morbidity were observed in any of the animals administered with CGA-7. There were no gross lesions or clinical signs related to extract administration (Supplementary File 2). All animals showed a normal body weight gain at the end of the experiment (Table 1). Based on the results obtained, it was concluded that CGA-7 falls under 'Category 5 or unclassified' according to the Globally Harmonized System (GHS) for the classification of chemicals. The cut-off LD50 value was greater than 2000 mg/kg.

Sub-chronic toxicity

A 90-day oral intake of CGA-7 up to 1000 mg/kg dose did not cause any toxic symptoms in rats of either sex. Daily observation of animals for physical and behavioural changes indicated no obvious signs of toxicity. Furthermore, necropsy findings revealed no abnormalities in the rats. The rats treated with different doses of CGA-7 did not show significant change in the water consumption as compared to control group (Figure 1). There was a significant increase in the feed intake of male rats of CGA-7 treatment groups during the study as compared to control ($p < 0.05$). On the contrary, the female rats showed significant reduction in food consumption at 500 and 1000 mg/kg doses of CGA-7 ($p < 0.01$; Table 2). Weekly increase in body weight gains followed a normal trend with insignificant changes compared to control (Figure 2).

The absolute organ weights of treated animals are shown in Table 3. The absolute organ weights of CGA-7–treated male rats were within the normal ranges and did not significantly vary compared to control. The absolute brain weights of female rats at 250, 500 ($p < 0.05$), and 1000 mg/kg reversal groups ($p < 0.01$) were significantly higher than the control group. The absolute spleen weights of CGA-7–treated female rats were increased significantly as compared to the control rats.

Table 4 shows the relative organ weight of experimental groups. The relative brain weight of the male rats in test reversal group was significantly reduced in comparison with control rats ($p < 0.05$). The relative brain weights at 500 ($p < 0.01$) and 1000 mg/kg ($p < 0.05$) extract-treated female rats were significantly increased as compared to control group. Also, the relative spleen weights in 500 ($p < 0.001$) and 1000 mg/kg ($p < 0.05$) extract-treated female rats were significantly higher compared to control rats.

The effect of 90-day treatment with CGA-7 on haematological parameters is presented in Table 5. Except for a significant change in few parameters, the haematological

Table 2. Effect of CGA-7 administration on feed intake in rats.

Male	Control	CGA-7 extract (mg/kg)			
		250	500	1000	1000 Reversal
Week 1	116.7 ± 1.28	90.3 ± 1.51***	124.7 ± 1.08	99.8 ± 0.48	91.9 ± 0.90***
Week 2	135.2 ± 3.15	148.6 ± 1.83	155.1 ± 2.31**	138.9 ± 1.64	144.7 ± 5.60
Week 3	122.4 ± 4.61	107.4 ± 2.44	127.0 ± 5.47	103.9 ± 2.40*	139.2 ± 3.80
Week 4	127.0 ± 4.35	132.1 ± 6.70	118.9 ± 4.90	161.5 ± 3.04***	150.7 ± 4.31***
Week 5	128.9 ± 2.80	113.9 ± 3.35	138.5 ± 6.10	132.6 ± 5.17	151.0 ± 4.40**
Week 6	124.5 ± 2.61	119.5 ± 4.93	124.7 ± 7.62	122.5 ± 4.80	146.6 ± 4.46**
Week 7	129.9 ± 3.46	124.1 ± 9.63	151.9 ± 4.94**	140.8 ± 8.50	122.0 ± 11.95
Week 8	148.9 ± 2.73	141.7 ± 2.88	136.1 ± 2.86	164.6 ± 1.41	150.4 ± 3.20
Week 9	136.7 ± 3.97	141.1 ± 2.76	154.2 ± 2.49*	141.6 ± 2.19	168.4 ± 1.60***
Week 10	145.6 ± 2.32	144.0 ± 3.37	150.0 ± 2.78	163.8 ± 3.04*	159.5 ± 1.92
Week 11	155.0 ± 3.24	139.0 ± 4.31	149.1 ± 2.10	169.9 ± 1.36	144.6 ± 1.88
Week 12	138.4 ± 4.03	144.6 ± 2.27	146.6 ± 3.95	146.9 ± 1.27	128.5 ± 4.90
Week 13	135.3 ± 2.98	137.6 ± 2.62	131.6 ± 4.31	130.3 ± 4.55	128.8 ± 2.92
Week 14	–	–	–	–	157.3 ± 2.75
Week 15	–	–	–	–	154.9 ± 1.74
Female					
Week 1	155.8 ± 3.01	145.2 ± 4.86	132.1 ± 1.81***	144.0 ± 2.69	146.0 ± 3.46
Week 2	69.85 ± 4.17	78.74 ± 2.13	74.06 ± 2.61	71.62 ± 2.39	81.14 ± 2.67
Week 3	129.6 ± 2.34	148.4 ± 4.50**	120.5 ± 3.72	123.1 ± 6.90	102.0 ± 4.75***
Week 4	119.9 ± 3.15	103.4 ± 2.76*	88.26 ± 2.33***	83.64 ± 2.36***	98.16 ± 4.10***
Week 5	102.2 ± 1.66	119.4 ± 1.45**	97.17 ± 1.75	98.68 ± 2.43	108.6 ± 4.42
Week 6	103.6 ± 1.32	106.9 ± 1.18	88.68 ± 1.30*	91.44 ± 1.59	107.7 ± 2.11
Week 7	101.7 ± 3.63	89.29 ± 1.20	73.84 ± 3.53***	49.82 ± 0.91***	97.45 ± 0.59
Week 8	110.1 ± 6.63	107.7 ± 1.12	86.23 ± 1.99***	92.03 ± 1.98**	100.2 ± 1.64
Week 9	135.2 ± 3.63	142.5 ± 3.69	104.2 ± 1.29***	116.9 ± 5.66**	130.8 ± 5.89
Week 10	140.4 ± 3.74	128.5 ± 3.61	103.2 ± 1.22***	110.1 ± 3.47***	125.0 ± 5.67*
Week 11	125.3 ± 3.25	121.6 ± 5.84	115.0 ± 3.44	111.0 ± 2.64	138.8 ± 6.12
Week 12	152.1 ± 3.92	142.9 ± 4.59	118.9 ± 3.80***	112.0 ± 3.39***	135.4 ± 3.98*
Week 13	125.7 ± 6.29	107.5 ± 2.70**	100.5 ± 3.76***	102.7 ± 3.15***	118.0 ± 4.24
Week 14	–	–	–	–	114.3 ± 2.11
Week 15	–	–	–	–	112.2 ± 4.14

Values are expressed as mean ± SEM ($n = 10$ for each group).
The data were analyzed using two-way ANOVA followed by Bonferroni post test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. control.

measures were marginally varied from the control. A significant reduction in lymphocytes and increase in neutrophils was observed in 250 mg/kg CGA-7–treated male rats compared to control ($p < 0.05$). The eosinophil count was significantly reduced in female rats treated with 1000 mg/kg CGA-7 ($p < 0.05$).

Serum biochemical analysis revealed no significant alteration in the evaluation parameters of CGA-7–treated male rats compared to the control animals (Table 6). The total bilirubin level was significantly increased in 1000 mg/kg CGA-7–treated female rats compared to control ($p < 0.05$). Furthermore, the female rats of 250 mg/kg treatment group showed significant increase in triglycerides ($p < 0.05$). None of the liver and kidney function parameters were significantly changed with reference to control group.

Table 7 shows the historical control data for the statistically significant parameters. It is suggestive thereof that the changes in haematological, biochemical parameters and relative organ weights were incidental due to the changes being within the physiological ranges. All the statistically significant changes, therefore, were considered toxicologically insignificant.

Histological examination of organs

Histopathological examination of vital organs from the treatment groups showed normal structure and absence of any gross pathological lesions (Table 8). The brain histology showed normal architecture in male and female rats with neuronal cells and glial cells. Hepatocytes were observed to be normal in the CGA-7–treated group. Normal histology of myocardial fibres was observed in the rats. Furthermore, rats treated with different doses of CGA-7 showed normal spleen histological pattern (white and red pulp were observed).

Kidney histology showed normal glomeruli, tubules, and vessels. In CGA-7–treated male rats, histological observation revealed testis showing normal histological features: seminiferous tubule, interstitial cell of Leydig, Sertoli cells, and spermatogonia. The 1000 mg/kg CGA-7 did not alter the histology of ovaries in rats. Ovaries showing the presence of follicles, normal vascularity, compact stroma, and intact germinal epithelium were observed. Figures 3 and 4 show the representative images of histopathological examination of vital organs in the control and 1000 mg/kg CGA-7 treatment groups.

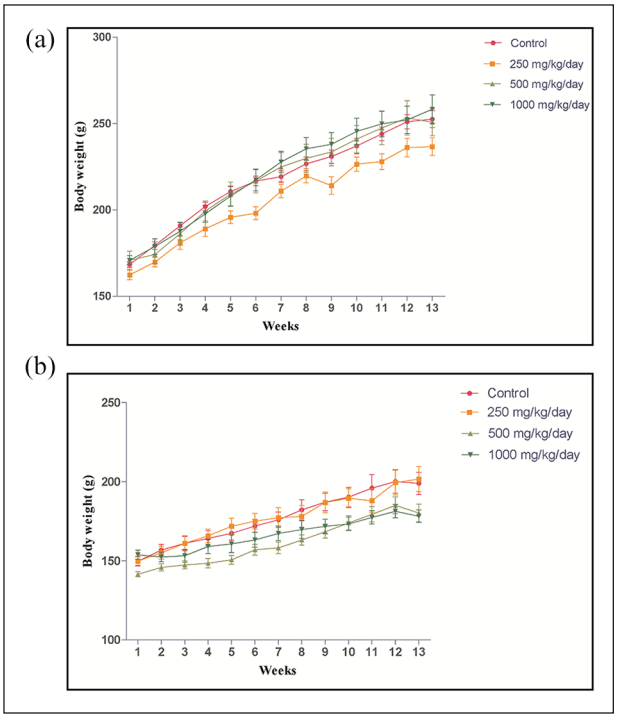

Figure 2. Mean body weight of (a) male and (b) female rats receiving CGA-7 extract for 90 days.
Values are expressed as mean ± SEM ($n = 10$ for each group).
*p value of <0.05 was considered significant using one-way ANOVA followed by Dunnet's t test. * denotes significant difference compared to control.

Discussion

Green coffee beans containing CGA is documented for various health benefits. Previously, the safety of several preparations from coffee beans such as diterpenoid-rich green coffee bean oil, roasted coffee extract, and fermented green coffee extract in experimental models have been reported.[18–20] However, there are no reports available on the toxicological studies of green coffee bean extract. In this study, we have evaluated the toxicity of CGA-7, standardized green coffee bean extract, in Wistar rats. First, the single dose acute oral toxicity was performed where CGA-7 at the dose of 2000 mg/kg did not exert any clinical signs of toxicity.

Furthermore, we have demonstrated no toxic effect of CGA-7 administered at 250, 500, and 1000 mg/kg/day to male and female rats for 90 days. Sub-chronic toxicity in rodents provides valuable information on the possible undesirable effects of administration of compounds or plant extracts.[21] In addition, these toxicology data are important to fix the appropriate dosage for long-term studies.[22]

In this study, 90-day administration of CGA-7 significantly affected the food consumption. The feed intake was observed to be increased in male rats, while it was markedly reduced in female rats compared to control. However, these significant changes were not observed consistently throughout the study. It is known that the changes in food and water consumption is associated with altered metabolism, which in turn reflected in the body weight changes.[23] In this study, there was no significant influence of CGA-7 on the normal metabolism as evident from the non-significant changes in body weight of animals.

Table 3. Absolute organ weights (g) of rats treated with CGA-7.

Sex	Organ	Control	CGA-7 extract (mg/kg)			
			250	500	1000	1000 R
Male	Brain	1.89 ± 0.07	1.84 ± 0.06	1.89 ± 0.07	1.84 ± 0.07	1.85 ± 0.07
	Heart	0.94 ± 0.02	0.95 ± 0.04	1.02 ± 0.05	0.99 ± 0.05	1.01 ± 0.04
	Liver	8.31 ± 0.28	8.15 ± 0.24	8.35 ± 0.34	8.79 ± 0.36	8.75 ± 0.26
	Spleen	0.79 ± 0.03	0.77 ± 0.04	0.87 ± 0.05	0.87 ± 0.04	0.91 ± 0.03
	Kidneys	1.73 ± 0.03	1.76 ± 0.05	1.77 ± 0.08	1.79 ± 0.07	1.72 ± 0.04
Female	Brain	1.39 ± 0.05	1.71 ± 0.05*	1.72 ± 0.07*	1.63 ± 0.06	1.75 ± 0.12**
	Heart	0.61 ± 0.02	0.75 ± 0.03*	0.65 ± 0.02	0.62 ± 0.03	0.71 ± 0.06
	Liver	5.36 ± 0.27	5.99 ± 0.27	5.55 ± 0.12	5.22 ± 0.24	5.75 ± 0.40
	Spleen	0.57 ± 0.03	0.74 ± 0.03***	0.80 ± 0.03***	0.70 ± 0.02**	0.59 ± 0.03
	Kidneys	1.05 ± 0.05	1.15 ± 0.04	1.10 ± 0.04	1.01 ± 0.04	1.16 ± 0.06

Values are expressed as mean ± SEM ($n = 10$ for each group).
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ were considered significant using one-way ANOVA followed by Dunnet's t test. * denotes significant difference compared to control.

Table 4. Summary of relative organ weights of rats treated with CGA-7.

Sex	Organ	Control	CGA-7 extract (mg/kg)			
			250	500	1000	1000 R
Male	Brain	0.74 ± 0.01	0.78 ± 0.01	0.75 ± 0.02	0.71 ± 0.01	0.67 ± 0.01*
	Heart	0.37 ± 0.01	0.40 ± 0.01	0.40 ± 0.02	0.38 ± 0.01	0.37 ± 0.01
	Liver	3.28 ± 0.10	3.44 ± 0.13	3.32 ± 0.12	3.39 ± 0.16	3.19 ± 0.12
	Spleen	0.31 ± 0.01	0.32 ± 0.01	0.35 ± 0.00	0.34 ± 0.02	0.33 ± 0.02
	Kidneys	0.68 ± 0.02	0.74 ± 0.01	0.70 ± 0.02	0.69 ± 0.02	0.63 ± 0.01
Female	Brain	0.76 ± 0.08	0.89 ± 0.03	1.01 ± 0.02**	0.96 ± 0.02*	0.92 ± 0.02
	Heart	0.33 ± 0.04	0.39 ± 0.02	0.38 ± 0.01	0.37 ± 0.01	0.37 ± 0.01
	Liver	2.92 ± 0.35	3.12 ± 0.13	3.27 ± 0.13	3.10 ± 0.11	3.02 ± 0.10
	Spleen	0.31 ± 0.037	0.39 ± 0.027	0.47 ± 0.02**	0.42 ± 0.02*	0.31 ± 0.01
	Kidneys	0.57 ± 0.06	0.60 ± 0.02	0.64 ± 0.01	0.60 ± 0.01	0.61 ± 0.02

Values are expressed as mean ± SEM ($n = 10$ for each group).
*$p < 0.05$ and **$p < 0.01$ were considered significant using one-way ANOVA followed by Dunnet's t test. * denotes significant difference compared to control.

Table 5. Effect of CGA-7 extract on haematological parameters in rats.

Male	Unit	Control	CGA-7 extract (mg/kg)			
			250	500	1000	1000 Reversal
Haemoglobin	g/dL	17.43 ± 0.31	16.83 ± 0.19	17.28 ± 0.15	16.81 ± 0.20	18.04 ± 0.21
RBC	10^6/μL	8.89 ± 0.13	8.74 ± 0.09	8.87 ± 0.11	8.52 ± 0.15	8.50 ± 0.58
HCT	%	40.69 ± 0.70	38.76 ± 0.71	39.61 ± 0.36	38.86 ± 0.48	39.70 ± 0.61
Reticulocytes	%	2.96 ± 0.06	2.71 ± 0.03	2.81 ± 0.05	3.08 ± 0.23	2.70 ± 0.03
MCV	fL	45.85 ± 0.58	44.08 ± 0.43	44.73 ± 0.56	45.71 ± 0.53	46.75 ± 0.56
MCH	Pg	19.56 ± 0.21	19.17 ± 0.10	19.43 ± 0.14	19.71 ± 0.23	20.17 ± 0.21
MCHC	g/dL	42.80 ± 0.45	43.62 ± 0.46	43.60 ± 0.38	43.22 ± 0.35	42.41 ± 0.35
Platelets	10^3/μL	400.4 ± 9.27	435.9 ± 41.79	410.2 ± 11.62	351.2 ± 13.99	361.8 ± 18.47
WBC	10^3/μL	9.92 ± 0.578	12.18 ± 1.39	10.32 ± 0.35	9.80 ± 0.72	12.56 ± 1.15
Lymphocytes	%	84.50 ± 1.08	78.82 ± 2.86*	82.08 ± 0.69	79.92 ± 1.17	87.05 ± 1.01
Monocytes	%	4.58 ± 0.23	5.21 ± 0.60	5.29 ± 0.25	5.60 ± 0.27	4.32 ± 0.11
Neutrophils	%	8.95 ± 0.83	13.73 ± 2.07*	10.36 ± 0.51	12.08 ± 0.96	6.77 ± 0.87
Eosinophils	%	1.95 ± 0.10	2.21 ± 0.24	2.26 ± 0.10	2.38 ± 0.11	1.84 ± 0.04
Basophils	%	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Clotting time	Sec	75.30 ± 10.56	86.50 ± 9.73	86.20 ± 9.78	57.30 ± 13.96	55.80 ± 8.17
Female						
Haemoglobin	g/dL	13.41 ± 0.63	13.84 ± 0.37	14.52 ± 0.60	13.78 ± 0.55	14.23 ± 0.20
RBC	10^6/μL	5.56 ± 0.21	5.82 ± 0.15	5.67 ± 0.40	5.78 ± 0.27	6.02 ± 0.11
HCT	%	30.27 ± 1.40	30.30 ± 0.91	31.59 ± 1.01	31.07 ± 1.41	31.69 ± 0.52
Reticulocytes	%	2.75 ± 0.31	2.04 ± 0.20	2.96 ± 0.50	3.57 ± 0.41	2.00 ± 0.21
MCV	fL	54.36 ± 0.66	52.07 ± 0.63	59.89 ± 7.59	53.88 ± 0.66	48.29 ± 0.51
MCH	pg	24.00 ± 0.47	23.70 ± 0.15	27.80 ± 4.26	23.86 ± 0.35	21.42 ± 0.26
MCHC	g/dL	44.25 ± 0.51	45.67 ± 0.30	45.80 ± 0.72	44.42 ± 0.60	44.52 ± 0.41
Platelets	10^3/μL	417.4 ± 57.21	414.6 ± 26.19	403.2 ± 29.24	363.5 ± 31.24	356.4 ± 18.07
WBC	10^3/μL	12.14 ± 2.68	9.950 ± 1.58	9.280 ± 0.73	9.820 ± 0.59	12.46 ± 1.23
Lymphocytes	%	87.42 ± 1.10	89.17 ± 1.13	88.62 ± 1.45	90.55 ± 1.06	83.57 ± 1.40
Monocytes	%	3.97 ± 0.25	3.53 ± 0.27	3.54 ± 0.38	2.94 ± 0.17	3.22 ± 0.32
Neutrophils	%	6.90 ± 0.84	5.78 ± 0.81	6.32 ± 0.96	5.25 ± 0.97	8.96 ± 1.00
Eosinophils	%	1.78 ± 0.10	1.49 ± 0.11	1.49 ± 0.16	1.24 ± 0.07*	2.22 ± 0.14
Basophils	%	0.03 ± 0.015	0.05 ± 0.016	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Clotting time	Sec	92.50 ± 9.67	77.90 ± 10.25	90.50 ± 9.28	90.90 ± 5.29	95.50 ± 17.30

CGA: chlorogenic acid; RBC: red blood cell; HCT: haematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBC: white blood cell.
Values are expressed as mean ± SEM ($n = 10$ for each group).
*$p < 0.05$ was considered significant using one-way analysis of variance (ANOVA) followed by Dunnet's t test. * denotes significant difference compared to control.

Table 6. Effect of CGA-7 extract on biochemical parameters in rats.

Male	Unit	Control	CGA-7 extract (mg/kg)			
			250	500	1000	1000 Reversal
ALT	IU/L	72.10 ±4.83	75.40 ± 4.05	62.80 ± 3.77	69.50 ± 5.78	65.00 ± 3.52
AST	IU/L	187.50 ± 12.69	183.30 ± 9.67	186.70 ± 13.57	188.80 ± 12.39	185.40 ± 6.40
ALP	IU/L	197.30 ± 25.01	191.10 ± 35.53	185.60 ± 10.52	199.90 ± 18.29	188.30 ± 8.55
Total protein	g/dL	7.58 ± 0.17	6.90 ± 0.49	7.25 ± 0.33	7.29 ± 0.13	7.44 ± 0.08
Albumin	mg/dL	3.89 ± 0.04	3.78 ± 0.03	3.85 ± 0.08	3.44 ± 0.35	3.85 ± 0.04
Glucose	mg/dL	138.60 ± 6.40	128.80 ± 3.34	128.60 ± 3.87	132.00 ± 4.19	131.20 ± 3.21
Total bilirubin	mg/dL	0.43 ± 0.06	0.52 ± 0.078	0.63 ± 0.16	0.44 ± 0.04	0.50 ± 0.33
Urea	mg/dL	55.02 ± 3.18	56.09 ± 3.03	64.45 ± 3.98	53.92 ± 3.55	60.68 ± 4.47
Creatinine	mg/dL	0.65 ± 0.08	0.58 ± 0.05	0.57 ± 0.02	0.55 ± 0.01	0.59 ± 0.02
Cholesterol	mg/dL	74.02 ± 3.27	64.00 ± 2.21	71.87 ± 5.27	70.51 ± 3.15	70.04 ± 1.47
Triglycerides	mg/dL	77.20 ± 9.02	77.36 ± 6.26	68.37 ± 4.61	68.07 ± 5.74	74.20 ± 5.68
HDL	mg/dL	46.22 ± 1.12	43.63 ± 1.58	44.65 ± 2.75	44.37 ± 1.62	49.25 ± 1.80
Calcium	mg/dL	9.71 ± 0.15	9.63 ± 0.62	9.42 ± 0.11	9.23 ± 0.09	9.17 ± 0.08
Phosphorous	mg/dL	4.75 ± 0.92	4.66 ± 1.17	5.60 ± 1.56	5.58 ± 2.43	4.41 ± 0.71
Female						
ALT	IU/L	72.20 ± 3.12	62.60 ± 1.88	67.70 ± 3.01	77.80 ± 3.26	70.10 ± 4.07
AST	IU/L	208.50 ± 5.66	189.10 ± 9.39	188.60 ± 7.14	185.20 ± 8.06	193.40 ± 7.19
ALP	IU/L	182.00 ±28.93	110.80 ±13.55	181.90 ±25.62	181.30 ± 17.02	176.30 ± 10.09
Total protein	g/dL	6.81 ±0.12	6.40 ± 0.11	6.63 ± 0.17	7.04 ± 0.19	7.16 ± 0.15
Albumin	mg/dL	3.83 ± 0.07	4.00 ± 0.06	3.79 ± 0.08	3.87 ± 0.06	3.97 ± 0.09
Glucose	mg/dL	113.40 ± 7.98	93.21 ± 7.12	102.30 ± 5.77	112.80 ± 11.41	119.30 ± 3.78
Total bilirubin	mg/dL	0.53 ± 0.05	0.89 ± 0.12	0.72 ± 0.08	1.04 ± 0.24*	0.71 ± 0.06
Urea	mg/dL	41.07 ± 2.90	37.23 ± 2.67	42.36 ± 1.50	40.74 ± 2.29	45.93 ± 3.55
Creatinine	mg/dL	0.58 ± 0.02	0.64 ± 0.02	0.59 ± 0.01	0.54 ± 0.01	0.57 ± 0.02
Cholesterol	mg/dL	57.33 ± 3.18	66.53 ± 5.07	61.78 ± 3.67	64.64 ± 3.61	67.66 ± 4.25
Triglycerides	mg/dL	49.43 ± 4.94	70.84 ± 5.75*	58.09 ± 5.64	54.94 ± 4.85	55.76 ± 4.11
HDL	mg/dL	44.17 ± 1.98	49.79 ± 3.50	45.41 ± 2.53	54.94 ± 2.34	55.21 ± 4.26
Calcium	mg/dL	9.25 ± 0.14	9.65 ± 0.07	9.52 ± 0.14	9.47 ± 0.10	9.51 ± 0.09
Phosphorous	mg/dL	14.50 ± 3.23	18.80 ± 3.66	19.78 ± 3.56	18.62 ± 5.63	19.85 ± 0.77

CGA: chlorogenic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; HDL: high-density lipoprotein.
Values are expressed as mean ± SEM ($n = 10$ for each group).
*$p < 0.05$ was considered significant using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. * denotes significant difference compared to control.

Table 7. Historical control data for statistically significant haematological and biochemical parameters.

Parameter	Historical control values (mean values)	Historical control ranges
Haematology		
Lymphocytes – male (%)	81.6	76.7–90.3
Neutrophils – male (%)	10.56	8.57–14.23
Eosinophils – female (%)	1.58	1.05–2.36
Clinical chemistry		
Total bilirubin – female (mg/dL)	0.74	0.48–1.10
Triglycerides – female (mg/dL)	56.8	45.4–68.6
Organ weights		
Brain – absolute weight (g) – female	1.52	1.35–1.65
Heart – absolute weight (g) – female	0.62	0.58–0.65
Spleen – absolute weight (g) – female	0.64	0.56–0.70
Brain – relative weight – male	0.75	0.68–0.78
Brain – relative weight – female	0.92	0.74–0.98
Spleen – relative weight – female	0.38	0.3–0.45

Table 8. Summary of histopathology findings.

Microscopic lesions	Group no. and dose (mg/kg/day)							
	G1 and 0		G2 and 250		G3 and 500		G4 and 1000	
	Male	Female	Male	Female	Male	Female	Male	Female
No. of animals	10	10	10	10	10	10	10	10
Brain								
Examined	5	5	5	5	5	5	5	5
No remarkable findings	5	5	5	5	5	5	5	4
Disruption of neuronal cell layers	0	0	0	0	0	0	0	0
Pyknotic nuclei	0	0	0	0	0	0	0	0
Hippocampus – focal gliosis	0	0	0	0	0	0	0	1
Hippocampus – apoptotic bodies	0	0	0	0	0	0	0	0
Hippocampus necrotic changes	0	0	0	0	0	0	0	0
Liver								
Examined	5	5	5	5	5	5	5	5
No remarkable findings	5	5	5	5	4	5	5	4
Mononuclear cell infiltration	0	0	0	0	1	0	0	0
Focal inflammations	0	0	0	0	0	0	0	1
Focal biliary hyperplasia	0	0	0	0	0	0	0	0
Congestion	0	0	0	0	0	0	0	0
Haemorrhage	0	0	0	0	0	0	0	0
Vacuolar changes and inflammation	0	0	0	0	0	0	0	0
Central vein – focal necrosis	0	0	0	0	0	0	0	0
Kidneys								
Examined	5	5	5	5	5	5	5	5
No remarkable findings	5	4	5	4	4	5	5	4
Tubular swelling	0	0	0	0	0	0	0	0
Interstitial fibrosis	0	0	0	0	0	0	0	0
Glomerulonephritis	0	0	0	0	0	0	0	0
Focal/mild inflammations	0	1	0	0	1	0	0	1
Tubular necrosis and fibrosis	0	0	0	1	0	0	0	0
Heart								
Examined	5	5	5	5	5	5	5	5
No remarkable findings	5	5	5	5	5	5	5	5
Focal/mild inflammations	0	0	0	0	0	0	0	0
Mild necrosis	0	0	0	0	0	0	0	0
Spleen								
Examined	5	5	5	5	5	5	5	5
No remarkable findings (normal splenic architecture)	5	5	5	5	5	5	5	5
Testes								
Examined	5	N/A	5	N/A	5	N/A	5	N/A
No remarkable findings	5	N/A	5	N/A	5	N/A	5	N/A
Ovaries								
Examined	N/A	5	N/A	5	N/A	5	N/A	5
No remarkable findings	N/A	5	N/A	4	N/A	5	N/A	4
Mild vascular changes	N/A	0	N/A	1	N/A	0	N/A	1

N/A: not applicable.

Nevertheless, there was a significant change in the relative brain weight of 250 and 500 mg/kg CGA-7 treatment groups, the absolute weights of these groups were insignificant compared to control group. The significant increase in the absolute and relative organ weights of spleen in female rats treated with 500 and 1000 mg/kg could suggest possible toxic effects of CGA-7 on these organs. However, these changes were well-within the historical control range. Splenic enlargement may result due to inflammation, haematopoiesis, or erythrophagocytosis.[24] There were no significant haematological changes

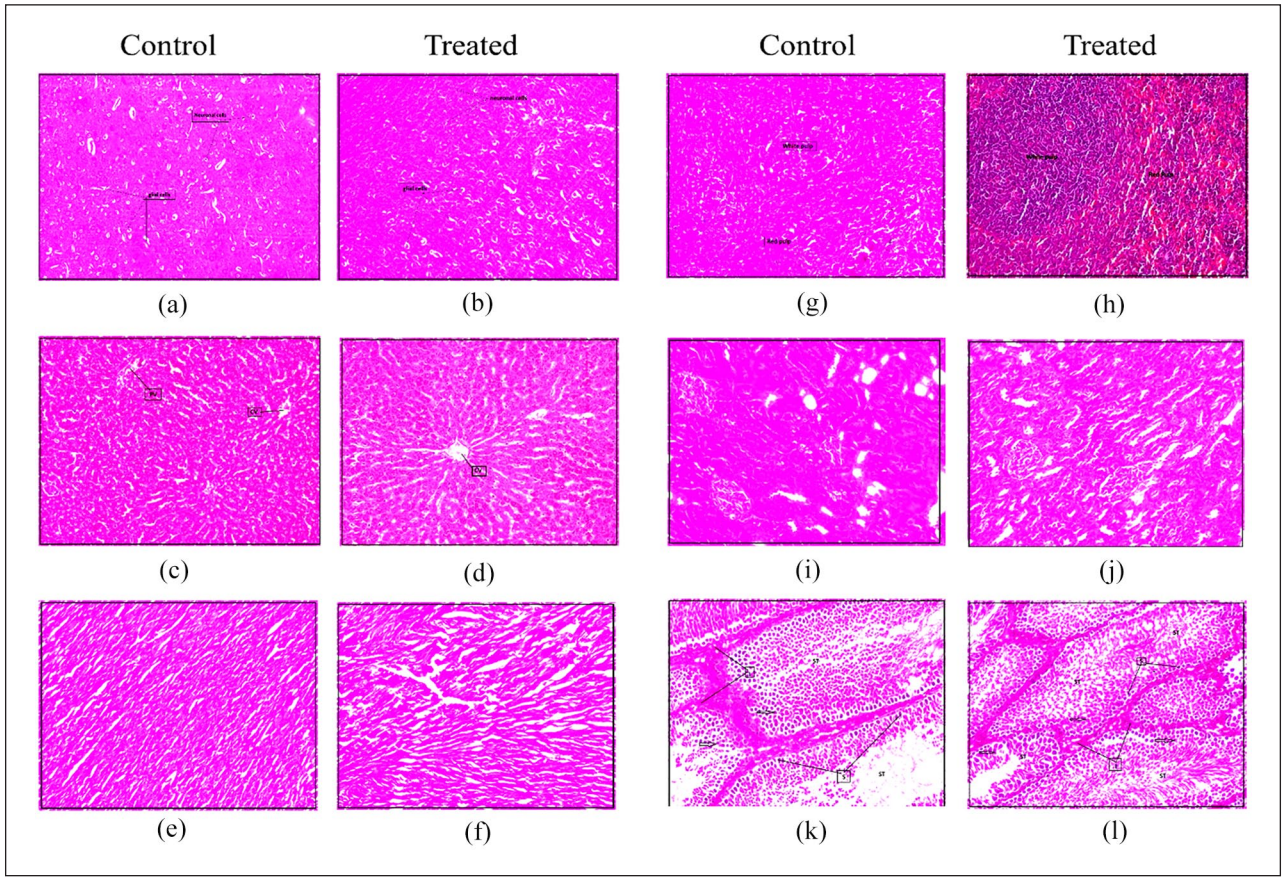

Figure 3. Effect of 1000 mg/kg CGA-7 on histology of vital organs of male rats. (a and b) brain, (c and d) liver, (e and f) heart, (g and h) spleen, (i and j) kidney, and (k and l) testes. Paraffin-embedded sections were stained with haematoxylin and eosin (HE; magnification: 100×).

correlating the splenic enlargement indicating that the changes were toxicologically insignificant.

Haematological assessment is an important aspect of toxicity study as it predicts the plausible human toxicity when translated, and provides useful information on inflammatory response, necrosis, and other infections.[25–28] Low haemoglobin, red blood cells (RBCs), and haematocrit are associated with anaemia.[29] CGA-7 at the tested doses did not significantly alter these parameters compared to control. Also, the subsequent measures such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were marginally changed in the CGA-7 treatment groups indicating that the extract does not affect erythropoiesis and morphology of erythrocytes in rats. Our results also indicate that CGA-7 does not affect the RBC producing ability of bone marrow.[30] However, the markers of immune response such as lymphocytes, neutrophils, and monocytes of male and female rats were significantly altered variably in different test doses of CGA-7. These changes did not follow a dose-dependent trend and hence not conclusive of possible effect of CGA-7 on immune response in rats. It is generally observed that variations in haematological and biochemical parameters are gender dependent.[31] Similarly, there was difference in haematological measures between the sexes. However, administration of CGA-7 did not induce any significant change in the haematological measures between male and female rats.

Biochemical measures of liver and renal function were not significantly altered in male and female rats after CGA-7 treatment. Although there were significant changes observed in total bilirubin and serum triglycerides of CGA-7–treated female rats compared to control, the values were within the historical control range. Elevated levels of ALT, AST, and ALP are indicative of liver damage.[32] In this study, ALT and ALP were reduced at 500 mg/kg CGA-7 treatment in male rats. Data from this study clearly indicate that ingestion of CGA-7 does not affect the normal hepatocyte function. Previously, it has been reported that males are susceptible to drug-induced liver injury than females.[33,34] In this study, no significant differences were observed in the liver function parameters of rats from either sex. At higher doses, the changes in TG levels were insignificant. Interestingly, the HDL-c level was significantly increased at 1000 mg/kg

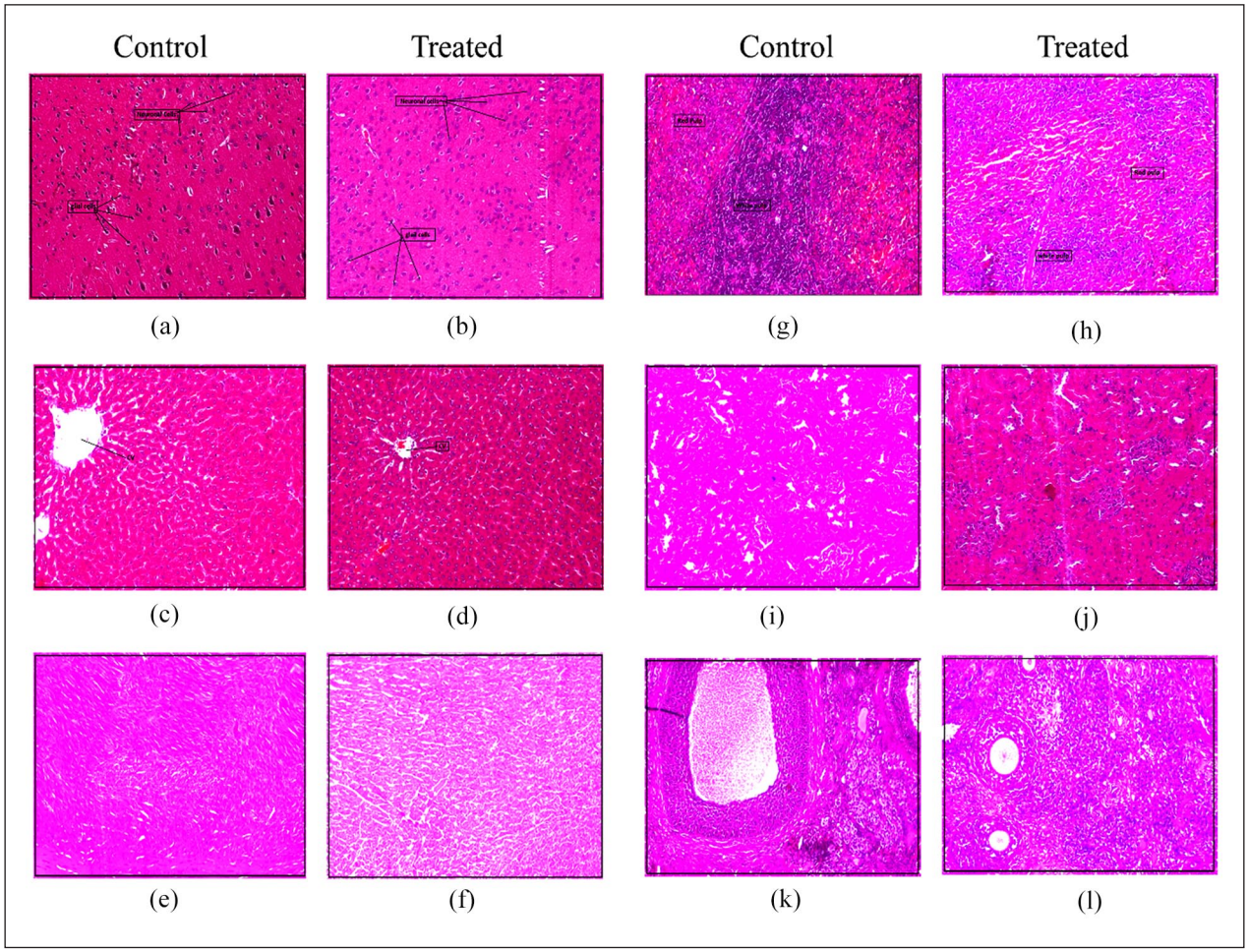

Figure 4. Effect of 1000 mg/kg CGA-7 on histology of vital organs of female rats. (a and b) brain, (c and d) liver, (e and f) heart, (g and h) spleen, (i and j) kidney, and (k and l) ovaries. Paraffin-embedded sections were stained with haematoxylin and eosin (HE; magnification: 100×).

CGA-7 treatment compared to control. Alterations in the parameters such as creatinine, urea, and uric acid from normal ranges reflect the renal problems and nephron dysfunction.[21,35] In this study, there was no significant change in creatinine and urea levels at any tested doses of CGA-7.

The macroscopic examination of organs did not reveal any hypertrophy or signs of injury. Histopathological findings suggested that the administration of CGA-7 did not induce abnormal changes in the cellular architecture of vital organs. Though there was a significant increase in relative organ weights of brain and spleen of CGA-7–treated rats, no histological signs of organ toxicity were observed in these vital organs. These results further confirm that the observed changes were incidental and hence not toxicologically significant.

In the present study, the decaffeinated coffee bean extract with higher CGA content did not show mortality or any adverse effects. On the contrary, in a previous study from Costa Silva Faria et al.,[36] the acute oral administration of CGA-rich hydroalcoholic fruit extract (CGA > 20%) at 5000 mg/kg in mice resulted in mortality. At the dose of 2500 mg/kg, stimulating effects on the autonomic system were noticed possibly due to the caffeine intoxication in the extract.[36]

Though this study provides preliminary data on the safety of a standardized green coffee bean extract, there are certain limitations in the conduct of the study. Here, we have not performed the power analysis to determine the sample size estimation. The number of animals per group was selected based on OECD guidelines.

Conclusion

Oral administration of CGA-7 at different doses did not induce any toxic signs or physiological changes in rats. It can be concluded based on our findings that CGA-7, the standardized green coffee bean extract, is considered as safe for oral ingestion.

Animal welfare

The present study followed international, national and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

Author contributions

All the authors have read and approved the article. S.K. was involved in the conceptualization and supervision of the article; S.H.V. contributed towards the experimental design, review and editing of the article; V.K. performed the animal experiments and was also involved in writing and original draft preparation.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from Institutional Animal Ethics Committee, Vidya Herbs Pvt Ltd (VHPL/PCL/IAEC/02/13).

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Supplemental material

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