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ACUTE AND SUB ACUTE TOXICITY STUDIES WITH GINGER EXTRACT IN RATS

Merina Benny [*1], M. R. Shylaja [2], Benny Antony [1], Nishant Kumar Gupta [1], Reshma Mary [1], Anjali Anto [2] and Sherina Jacob [1]

Research and Development Laboratory [1], Arjuna Natural Private Ltd., Erumathala PO, Aluva - 683112, Kerala, India.
Centre for Plant Biotechnology & Molecular Biology [2], IT-BT Complex, College of Horticulture, Kerala Agricultural University, Thrissur - 680656, Kerala, India.

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Correspondence to Author:
Merina Benny

GM - QA & R&D,
Arjuna Natural Private Ltd.,
Erumathala PO, Aluva - 683112,
Kerala, India.

E-mail: research@arjunanatural.com

ABSTRACT: The dry ginger rhizome was extracted with ethyl acetate, and gingerol content was enriched by liquid-liquid extraction. The extract was analyzed for the percentage of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol by HPLC. The acute toxicity was evaluated as per OECD guidelines 423. Ginger extract was fed at 2000 mg/kg body weight to overnight fasted female rats (8-10 weeks old; 160-180 g). The animals were observed daily for clinical signs of abnormality/mortality. After 14 days, animals were sacrificed, and gross pathological changes were recorded. Sub-acute toxicity of ginger extract was studied by feeding the extract at 100, 500, and 1000 mg/kg daily to rats as per OECD guidelines 407. The total gingerols content in the purified viscous extract was 36-43%. The finished formulation of ginger extract used for the toxicity studies had 8% total gingerols. In the acute toxicity study, no mortality or clinical signs of toxicity were observed at a maximum recommended dose level of 2000 mg/kg; therefore, the LD50 is >2000 mg/kg in rats. The repeated administration of ginger extract for 28 days in rats at the maximum dose level of 1000 mg/kg did not induce any observable toxic effects when compared to its corresponding control animals. The hematology and biochemistry profile of treated rats was similar to control animals, and the difference was non-significant ($p>0.05$). The histopathology of major organs of all the control and treated animals was normal. In this study, the NOAEL (No Observed Adverse Effect Level) was calculated as 1000 mg/kg daily for rats.

INTRODUCTION: Ginger (*Zingiber officinale* Roscoe), belonging to the family Zingiberaceae, is one of the most commonly consumed dietary condiments in the world [1]. The oleoresin from the rhizome of ginger contains many bioactive components, such as [6]-gingerol, which is the most prominent pungent ingredient that is believed to exert a number of remarkable pharmacological and physiological activities.

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Ginger has been used for many years for the treatment of numerous ailments, such as nausea, colds, arthritis, hypertension, and migraines. The medicinal, chemical and pharmacological properties of ginger have been extensively reported in the literature [2-7].

Gingerols are the major constituents of fresh ginger rhizomes and are found slightly less in dry ginger, whereas the concentrations of shogaols, which are the major gingerol dehydration products, are more abundant in dry ginger than in fresh ginger [8]. About 31 gingerol-related constituents have been identified from the methanol extracts of fresh ginger rhizomes [9]. Koh and coworkers (2009) have reported about 14 biologically active compounds by fractionation of ginger extract.

These compounds include [4]-gingerol, [6]-gingerol, [8]-gingerol, [10]-gingerol, [6]-paradol, [14]- shogaol, [6] -shogaol, 1-dehydro-[10]-gingerdione, [10]-gingerdione, hexahydrocurcumin, tetrahydrocurcumin, gingerenone A, 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one, and methoxy-[10]-gingerol [10].

The use of natural or alternative medicines has increased markedly over the last few years. Especially the older adults are using complementary and alternative medicine, herbal remedies, and dietary supplements without advice from a physician on the assumption that these substances will have a positive effect [11]. Scientifically, this might not be a safe or advisable practice. A few toxicity studies with ginger are reported in animals. A patented ginger extract (25–100 mg/kg) with a high percentage of gingerols and shogaols did not induce significant changes in blood glucose, blood pressure, blood coagulation, and heart rate in normal male rats in acute toxicity study [12]. In another study, ginger extract at the dosages of 100, 333, and 1000 mg/kg administered to pregnant rats for 10 days during the period of organogenesis caused neither maternal nor developmental toxicity at any of the tested dosages [13].

However, ginger is reported to contain mutagenic and antimutagenic constituents [14]. It is reported that alcohol extract of ginger is more cytotoxic than aqueous extracts in cultured Dalton's lymphoma ascites tumor cells, human lymphocytes and Chinese Hamster Ovary cells, and Vero cells [15]. In humans, ginger may cause gastric reflux, and high dose ginger may act as a gastric irritant [16].

Although ginger is generally considered to be safe [17], the lack of a complete understanding of its mechanisms of action suggests caution in its therapeutic use [18]. The reports on toxicity studies indicate that potential toxicity of ginger cannot be ruled out, especially when ginger is consumed in higher dosages for long terms or in the form of purified extracts containing a high percentage of actives. The aim of the present study, therefore, was to assess the toxicity of a purified ginger extract in rats as per OECD (Organisation for Economic Co-operation and Development) guidelines to support its safety for human use.

MATERIALS AND METHODS:

Chemicals: The reference standards of 6-gingerol (CAS No. 23513-14-6; 98% pure), 8-gingerol (CAS No. 23513-08-8; 95% pure), 10-gingerol (CAS No. 23513-15-7; 95% pure) and 6-shogaol (CAS No. 555-66-8; 90% pure) were purchased from Sigma-Aldrich, Germany. Acetonitrile and methanol were of HPLC grade from Merck, Germany. The ethyl acetate was purchased from Merck, India, and purity was 98%. The water used in this study was ultrapure obtained from a Milli-Q RO system (Millipore Corporation, France). Ortho-phosphoric acid used for the experiment was of analytical grade and purchased from Merck Specialties Private Limited, Mumbai, India. All the other chemicals were of analytical grade and purchased locally.

Plant Material and Identification: The ginger rhizomes were collected from Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. The rhizomes were identified and authenticated by Dr. M. R. Shylaja, Kerala Agricultural University. The voucher specimen number GINGER-KR (Latin name: *Zingiber officinale*; Plant part: Rhizome) with Herbarium ID – HERB-ED-29 was deposited for future reference.

Extraction and Enrichment:

Preparation of Extract: The dry ginger rhizome of the ginger variety Karthika released from Kerala Agricultural University was used for the study. Five kilogram of dried ginger rhizome flakes with less than 10% moisture was extracted with 50 L of ethyl acetate at a steady temperature of 65 °C. The extraction was continued for about 3 h. On reaching the 5-6% TDS (total dissolved solids), the solvent part was separated from the residue using a polypropylene filter cloth. The residue was further extracted two more times with fresh ethyl acetate. The ethyl acetate portions were pooled and concentrated under vacuum and dried.

Enrichment of Gingerol Content: Fourty gram of ethyl acetate extract was purified further with 500 ml of 50% aqueous ethanol. In brief, ginger extract from the variety Karthika was blended with 50% ethanol and refluxed for 1 hr. The ethanol part was filtered out, and the residue was extracted two more times with 50% ethanol. All the ethanol parts were pooled, concentrated, and the yield of ethanol

extract was 50%. The ethanol extract was further extracted with ethyl acetate at 66.6 KPa and 50 °C steady temperature and pressure. The extraction was carried out two more times to achieve a better yield. All the ethyl acetate parts were pooled together and concentrated under a vacuum. A viscous liquid extract was obtained after the removal of the solvent.

Preparation of Powdered Ginger Extract: 11.4 g of enriched ginger extract (viscous liquid) was taken in a vessel and stirred uniformly. 9 g of Vitamin-E was added at a steady rate into the vessel till a uniform mixture was obtained. 18 g of gum acacia, 18.27 g of cellulose, and 280 mg of the anti-caking agent were dissolved in a separate vessel in 100 ml of alcohol. The homogenous mixture of ginger extract and Vitamin-E was added to the alcoholic solution of excipients. The solution was concentrated under a vacuum till the entire solvent was removed to get ginger extract in powdered form. The powdered extract was analyzed in HPLC and assured not less than 8% total gingerols. This standardized ginger powder extract was used for toxicity studies.

HPLC Analysis:

Instrumentation: Liquid chromatographic separation was performed on a binary HPLC (Waters) separation 2998 series equipped with variable wavelength photodiode array (PDA) detector module, autosampler 2707 with injection volume of 20 µl and 1525 pump. The column used was C_{18} Sunfire (150 × 4.6 mm, 5 µ particle size), and data was recorded using Empower 3 software.

The separation was carried out with a mobile phase consisting of acetonitrile, (0.1%) ortho-phospohoric acid in water and methanol (55:44:1, v/v/v) at a flow rate of 1.0 ml/min and detection monitored at 282 nm with PDA detector.

Preparation of Standard Solution: Standard stock solutions of 6-gingerol, 8-gingerol, 10-gingerol, and 6- shogaol were prepared by dissolving 10 mg of respective standards in 10 ml of methanol to get a stock solution containing 1000 µg/ml. From the stock solution, different aliquots were prepared to get known concentrations from 25 to 500 µg/mL. Calibration curves were constructed individually for 6, 8, 10 gingerols, and 6- shogaol by plotting the peak areas versus the concentrations of each analyte.

Preparation of Sample Solution: 125 mg of powdered ginger extract was weighed into a 25 ml volumetric flask and dissolved in methanol by sonication for 1 min. The solution was filtered through 0.2 µm syringe filter and injected directly into HPLC.

Animals: Sprague Dawley rats (8-10 weeks old) weighing 160-180 g were purchased from Small Animals Breeding Station, Veterinary University, Mannuthy, Kerala and maintained at 22±2 °C with 50-70% relative humidity and lighting was controlled to give 12 h artificial light (6 am-6 pm) each day. Filtered drinking water (Aquaguard) and pellet feed manufactured by M/s. Kerala feeds, Thrissur, India, was provided *ad libitum*. The study was approved by the Institutional Animals Ethics Committee of Arjuna Natural Private Ltd., Kochi, Kerala, India (1524/PO/RcBi/S/11/CPCSEA; Protocol ID: ANEL/IAEC/2016-I/1607017).

Acute Toxicity Study in Rats: This study was performed in accordance with the OECD guideline for the testing of chemicals, "Acute Oral Toxicity Study (Acute Toxic Class Method)", Guideline No. 423, adopted on December 17, 2001. Six female rats weighing 160-180 g were used in this study. The study was divided into two steps, and the animals were acclimatized for 7 days before the commencement of each step. The animals fasted overnight before and four hours after dosing. Taking three rats in the first step, a limit test was performed. The test substance (Ginger extract with 8% total gingerols) suspended in 0.5% Tween 80 (polysorbate 80) was freshly prepared and administered orally at the dose level of 2000 mg/kg body weight (2 ml/100 g body weight) with the help of a stainless steel cannula attached with a syringe in the first step. No mortality or toxic signs and symptoms were observed in any of the animals at the first step, hence to confirm the findings of the first step, the next step was performed by taking three more female rats, which were administered the same dose of 2000 mg/kg.

The treated rats were observed for clinical signs of abnormality/mortality five times on day 1 (day of administration), *i.e.,* at 30 min and four times at

hourly (post-administration) intervals and thereafter once daily for a total of 14 days. The body weights of rats were recorded on day 1, day 7, and day 14. No mortality or treatment-related toxic signs and symptoms were observed in the animals at both steps. As no toxic signs were noted, no further testing was required. After 14 days, animals were sacrificed, and gross pathological changes were recorded.

Sub-acute (28 days Repeated Dose) Toxicity Study: This study was conducted as per OECD guidelines for testing of chemicals (Guideline No. 407). One hundred rats (50 males/50 females) were divided into four groups of 20 animals (10 males and 10 females) in each and two groups (satellite/recovery groups) of 10 animals (5 males and 5 females) in each group. The animals were acclimatized for seven days before the commencement of dosing. Three groups of 20 rats each (10 male and 10 female) were administered with ginger extract (containing 8% total gingerols) suspended in 0.5% Tween 80, orally at the dosage levels of 100 mg/kg body weight (low dose; LD), 500 mg/kg body weight (medium dose; MD) and 1000 mg/kg body weight (high dose; HD) respectively for seven days a week for 28 days with the help of cannula attached with the syringe. The maximum volume of suspension administered was 1 ml/100 g body weight. Similarly, the fourth group of 20 rats (10 male and 10 female rats) were orally administered with 0.5% Tween 80 in distilled water (vehicle) for 28 days and was designated as a control group.

Two additional satellite (recovery) groups of 10 rats (5 male and 5 female) each were also kept and designated as 'Satellite control' and 'Satellite high dose' and were administered with vehicle and ginger extract (1000 mg/kg) respectively daily for 28 days. After the terminal sacrifice of the test and control group animals, both recovery group animals (satellite control and high satellite dose) were kept under observation for an additional 14 days to check the reversibility, persistence or delayed toxic effect, if any. The animals were observed daily for appearance, behavior, and toxic signs and symptoms. Blood was collected from retro-orbital sinus from all the animals before terminal sacrifice for detailed hematological and biochemical evaluation. All the major organs (liver, kidneys, lungs, heart, brain, pancreas, stomach, testes, uterus *etc*.) were collected and preserved in 10% formalin for histopathology.

Statistical Analysis: The data were analyzed by one-way analyses of variance (ANOVA) using GraphPad Prism software. Following ANOVA, Dunnett's pair-wise comparison of means of treated groups with control group mean was carried out individually. The data is presented as the Mean ± SEM (standard error of the mean), and p-value <0.05 was considered as significant.

RESULTS:

Extraction and HPLC Analysis: The yield of the ethyl acetate extract was about 5-6%. It was obtained as a viscous liquid extract, and total gingerols in this extract were about 16-18%. The yield of enriched extract was about 34%, and the total gingerols content of the enriched extract was about 36%-43%. The dried ginger extract used in the toxicity studies had total gingerols 8.27% (6-gingerol = 6.41%; 8-gingerol = 0.86%; 10-gingerol = 1.00%) and 6-shogaol 0.76%. Typical HPLC chromatograms for mixed standards and samples are shown as **Fig. 1-2**.

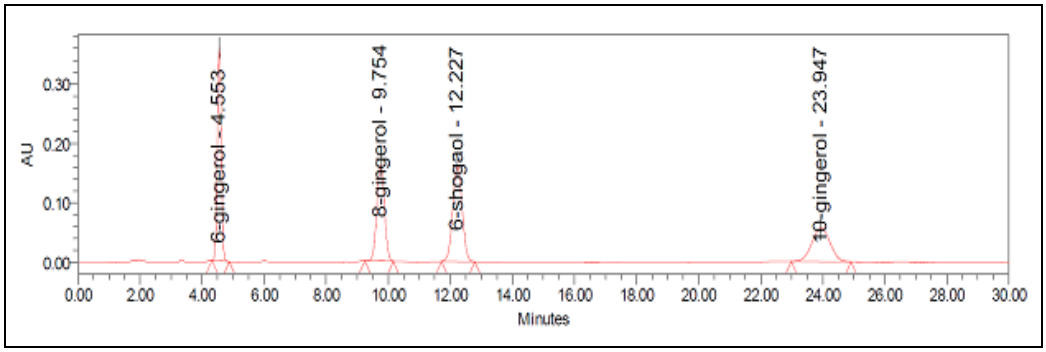

FIG. 1: HPLC CHROMATOGRAMS FOR MIXED STANDARDS OF 6-GINGEROL, 8-GINGEROL, 10-GINGEROL AND 6-SHOGAOL

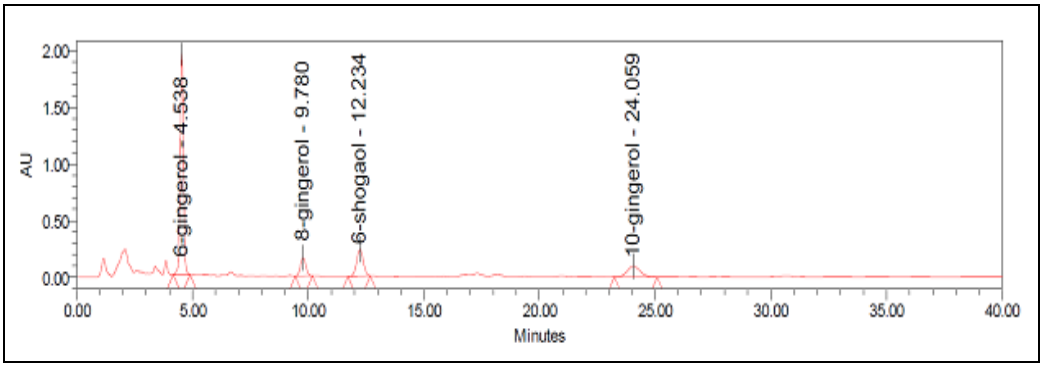

FIG. 2: HPLC CHROMATOGRAMS FOR GINGER EXTRACT SAMPLE

Acute Toxicity Study in Rats: No clinical signs of toxicity were observed in any of the treated rats at the dose level of 2000 mg/kg body weight of the ginger extract. No mortality was observed in the animals at step I and step II administered with the ginger extract at 2000 mg/kg body weight. Individual body weights were recorded prior to oral administration (day 1) and on days 7 and 14 following oral administration. The gain in body weights was normal in all the rat's **Table 1**. All animals were sacrificed at the end of the study and they did not reveal any abnormality of gross pathological significance. Based on these findings of the acute oral toxicity (Acute Toxic Class Method) of the ginger extract in rats, the LD_{50} of the extract may be classified as GHS (Globally Harmonized System) category 5 (LD_{50}>2000 mg/kg) as per OECD Guideline No. 423, December 2001.

TABLE 1: BODY WEIGHT, BODY WEIGHT CHANGES AND PRE-TERMINAL DEATHS IN ACUTE TOXICITY STUDY

Dose (mg/kg)	Rat no.	Sex	Bodyweight (g)					No. dead / No. tested
			Day 1	Day 7	Day 7 – Day 1	Day 14	Day 14 – Day 1	
2000	1	Female	162	174	12	178	16	0/6
	2	Female	165	175	10	179	14	
	3	Female	162	170	8	173	11	
	4	Female	164	176	12	180	16	
	5	Female	175	184	9	190	15	
	6	Female	172	183	11	188	16	

Sub-acute Toxicity Study: There were no treatment-related toxic sign and symptoms observed in low dose (100 mg/kg body weight), medium dose (500 mg/kg body weight), high dose (1000 mg/kg body weight), and recovery high dose (1000 mg/kg body weight) group animals of ginger extract when compared to their respective control animals. Body weights of all the control and test group animals were recorded weekly. Bodyweight gain of all the treatment groups and recovery group animals (LD, MD, HD & recovery HD) was comparable to their control counterparts. Feed consumption of the animals of low dose, intermediate dose, high dose, and high recovery dose was comparable to the control group and recovery control group animals.

There were no variations in the haematological parameters of animals of low dose (100 mg/kg), medium dose (500 mg/kg), high dose (1000 mg/kg), and high recovery dose (1000 mg/kg) groups when compared to the control group animals **Table 2-3**. Similarly, the biochemical parameters of animals of all the treatment groups, *i.e.*, low dose, intermediate dose, and high dose, were comparable to the biochemical parameters of the control group animals at the terminal sacrifice.

The biochemical parameters of the recovery high dose group were also comparable to its recovery control animals **Table 4-5**.

None of the animals died during the study in any of the treated groups as well as the control group. Organ weights of animals of all treatment groups were comparable to their respective control counterparts. There were no significant histopathological changes in the animals of LD, MD, and HD group when compared to their control counterparts **Fig. 3-4**. A summary of histopathological findings in male and female rats is presented in **Table 6-7**.

TABLE 2: HAEMATOLOGICAL PARAMETERS OF MALE RATS IN REPEATED DOSE 28-DAYS TOXICITY STUDY

Treatment	Dose (mg/kg)	Hb (gm/dl)	WBC ($\times10^3$/cmm)	N (%)	L (%)	E (%)	RBC (mill/cmm)	Platelets ($\times10^5$/cmm)	PCV (%)	MCV (FL)	MCH (pg)	MCHC (gm/dl)	RDW-SD	MPV	RDW-CV (%)	PDW	PCT (%)
Control	0	15.13	7.97	55.33	35.33	9.33	7.76	5.96	39.67	51.13	17.73	34.70	28.13	8.13	15.03	14.43	0.50
		±0.71	±0.92	±3.68	±2.23	±0.69	±0.51	±0.38	±2.14	±3.11	±0.87	±1.98	±1.56	±0.54	±0.79	±0.66	±0.03
Ginger extract	100	14.53	7.53	64.00	32.00	4.00	7.73	5.92	41.00	52.77	18.63	35.37	26.80	7.93	15.43	14.33	0.47
		±0.69	±0.96	±4.79	±1.96	±0.44	±0.59	±0.44	±2.54	±3.25	±0.96	±2.24	±1.58	±0.62	±0.78	±0.68	±0.03
Ginger extract	500	15.93	8.06	63.00	31.33	5.67	8.10	5.12	44.10	54.27	19.50	36.10	27.50	8.00	15.10	14.37	0.41
		±0.95	±1.05	±5.24	±2.05	±0.59	±0.66	±0.47	±2.36	±3.41	±1.14	±2.26	±1.62	±0.74	±0.85	±0.69	±0.02
Ginger extract	1000	14.90	10.40	56.67	38.33	5.00	8.17	4.51	42.03	51.33	18.17	35.43	26.17	8.37	15.17	14.87	0.38
		±0.98	±1.35	±4.25	±2.25	±0.78	±0.59	±0.36	±2.17	±3.65	±1.06	±2.84	±1.85	±0.87	±0.74	±0.74	±0.02
Recovery control	0	15.22	8.20	50.33	40.33	10.33	7.14	5.25	39.62	54.23	18.82	37.52	28.52	8.13	15.22	14.51	0.44
		±1.01	±0.78	±3.99	±2.63	±1.25	±0.48	±0.51	±2.68	±3.84	±1.08	±2.51	±1.96	±0.82	±0.72	±0.75	±0.02
Recovery high dose Ginger ext	1000	14.82	8.10	52.67	39.00	8.33	7.34	5.45	35.55	54.94	19.61	39.93	28.51	8.30	15.24	14.62	0.52
		±0.84	±1.02	±5.24	±2.48	±0.96	±0.46	±0.48	±2.37	±3.11	±0.99	±2.18	±1.87	±0.75	±0.77	±0.71	±0.03

Analysis of variance (ANOVA) was used for statistical analysis. Data are presented as the Mean ± SEM. P>0.05, compared with the respective control. No significant differences were observed between the test and control groups. Hb=Hemoglobin; N=Neutrophils; L=Lymphocytes; E=Eosinophils; PCV=Packed Cell Volume (Hematocrit); MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; RDW= Red Blood Cell Distribution Width; MPV= Mean Platelet Volume; PCT= Platelet crit

TABLE 3: HAEMATOLOGICAL PARAMETERS OF FEMALE RATS IN REPEATED DOSE 28-DAYS TOXICITY STUDY

Treatment	Dose (mg/kg)	Hb (gm/dl)	WBC ($\times10^3$/cmm)	N (%)	L (%)	E (%)	RBC (mill/cmm)	Platelets ($\times10^5$/cmm)	PCV (%)	MCV (FL)	MCH (pg)	MCHC (gm/dl)	RDW-SD	MPV	RDW-CV (%)	PDW	PCT (%)
Control	0	15.03	7.50	54.33	38.33	7.33	7.26	5.59	39.50	53.60	19.77	38.50	28.73	8.23	15.17	14.53	0.48
		±0.81	±0.93	±3.54	±5.41	±0.65	±0.63	±0.45	±2.89	±3.65	±1.08	±1.54	±1.41	±0.79	±0.88	±0.65	±0.02
Ginger extract	100	15.97	7.13	48.00	44.33	7.67	7.97	6.45	45.00	56.07	19.80	35.40	28.83	8.10	14.83	14.33	0.52
		±0.75	±0.87	±4.10	±3.44	±0.69	±0.57	±0.39	±3.14	±3.41	±1.06	±1.63	±1.63	±0.63	±1.04	±0.41	±0.03
Ginger extract	500	14.60	8.60	66.67	27.33	6.00	7.53	5.35	41.53	55.10	19.30	35.10	26.80	7.83	14.53	14.23	0.42
		±0.78	±0.84	±4.66	±4.33	±0.74	±0.85	±0.44	±3.05	±3.25	±0.98	±2.61	±1.98	±0.57	±0.63	±0.63	±0.02
Ginger extract	1000	14.80	7.96	53.67	40.00	6.33	7.50	4.85	40.40	53.40	19.50	36.57	27.13	8.03	14.43	14.47	0.39
		±0.74	±0.98	±3.85	±2.44	±0.77	±0.77	±0.47	±2.14	±3.98	±1.03	±2.47	±2.01	±0.55	±0.98	±0.89	±0.01
Recovery control	0	15.50	5.40	50.33	40.33	10.33	7.15	5.06	40.65	56.91	21.64	38.12	28.11	8.32	13.44	14.82	0.41
		±0.86	±0.87	±3.79	±2.98	±1.21	±0.69	±0.55	±2.45	±2.44	±1.04	±2.10	±1.77	±0.56	±0.71	±0.57	±0.02
Recovery high dose Ginger ext	1000	15.25	6.70	41.00	50.00	9.00	7.24	5.29	40.00	52.22	21.13	37.31	28.12	8.61	14.31	14.23	0.47
		±0.88	±0.66	±3.22	±3.11	±1.08	±0.58	±0.52	±2.63	±3.34	±1.25	±1.84	±1.81	±0.51	±0.56	±0.55	±0.02

Analysis of variance (ANOVA) was used for statistical analysis. Data are presented as the Mean ± SEM. P>0.05, compared with the respective control. No significant differences were observed between the test and control groups. Hb=Hemoglobin; N=Neutrophils; L=Lymphocytes; E=Eosinophils; PCV=Packed Cell Volume (Hematocrit); MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration; RDW= Red Blood Cell Distribution Width; MPV= Mean Platelet Volume; PCT= Platelet crit

TABLE 4: BIOCHEMICAL PARAMETERS OF MALE RATS IN 28 DAYS REPEATED DOSE TOXICITY STUDY

Treatment	Dose (mg/kg)	CHO (mg/dl)	TG (mg/dl)	ALKP (IU/L)	T.Bil (mg/dl)	D.Bil (mg/dl)	T.Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	FBS (mg/dl)
Control	0	71.00	62.00	138.67	0.67	0.13	6.70	3.33	3.33	0.97	99.00	77.33	25.47	0.73	79.00
		±3.48	±4.05	±11.54	±0.04	±0.01	±0.32	±0.18	±0.17	±0.05	±5.02	±3.88	±1.45	±0.03	±3.96
Ginger	100	69.33	57.00	153.00	0.60	0.10	6.33	3.23	3.10	1.00	88.00	73.33	22.67	0.63	81.67
extract		±3.96	±3.54	±12.08	±0.03	±0.00	±0.33	±0.16	±0.15	±0.04	±5.12	±5.24	±1.25	±0.04	±3.54
Ginger	500	66.00	64.67	149.33	0.50	0.10	7.07	3.27	3.80	0.83	91.67	78.33	20.67	0.60	80.33
extract		±3.14	±3.98	±12.45	±0.02	±0.00	±0.45	±0.14	±0.18	±0.05	±5.21	±5.96	±1.33	±0.08	±3.66
Ginger	1000	63.33	55.33	155.33	0.63	0.10	6.47	3.27	3.20	0.97	91.33	73.33	23.33	0.60	75.67
extract		±4.05	±2.65	±13.69	±0.03	±0.01	±0.65	±0.18	±0.17	±0.05	±6.32	±6.35	±1.05	±0.05	±3.55
Recovery	0	63.32	70.11	140.00	0.63	0.12	6.61	3.31	3.30	1.00	95.00	74.67	28.00	0.67	80.33
control		±3.97	±3.41	±12.88	±0.02	±0.00	±0.51	±0.19	±0.18	±0.05	±5.11	±5.41	±1.63	±0.07	±3.68
Recovery	1000	84.11	67.24	139.54	0.72	0.12	6.83	3.42	3.41	1.00	92.33	74.33	25.47	0.72	76.50
high dose		±4.55	±3.55	±12.97	±0.03	±0.01	±0.33	±0.18	±0.17	±0.04	±5.25	±6.21	±1.78	±0.08	±3.21
Ginger ext															

Analysis of variance (ANOVA) was used for statistical analysis. Data are presented as the Mean ± SEM. P>0.05, compared with the respective control. No significant differences were observed between the test and control groups. CHO=Cholesterol; TG=Triglycerides; ALKP=Alkaline phosphatase; T.Bil=Total Bilirubin; D.Bil=Direct Bilirubin; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic pyruvic transaminase; BUN= blood urea nitrogen; FBS=Fasting Blood Sugar

TABLE 5: BIOCHEMICAL PARAMETERS OF FEMALE RATS IN 28 DAYS REPEATED DOSE TOXICITY STUDY

Treatment	Dose (mg/kg)	CHO (mg/dl)	TG (mg/dl)	ALKP (IU/L)	T.Bil (mg/dl)	D.Bil (mg/dl)	T.Protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	FBS (mg/dl)
Control	0	82.33	60.00	138.33	0.67	0.10	6.87	3.33	3.27	1.00	97.67	74.33	24.40	0.70	78.67
		±4.12	±3.42	±11.62	±0.04	±0.00	±0.33	±0.18	±0.18	±0.05	±5.14	±4.21	±1.52	±0.03	±3.66
Ginger	100	80.33	50.67	150.00	0.60	0.10	7.07	3.40	3.73	0.90	93.67	69.00	23.33	0.87	77.67
extract		±5.24	±3.98	±12.08	±0.03	±0.00	±0.35	±0.19	±0.17	±0.04	±6.11	±4.66	±1.55	±0.04	±3.21
Ginger	500	75.67	67.00	154.33	0.63	0.10	6.97	3.23	3.73	0.80	94.67	68.00	17.33	0.87	74.33
extract		±4.65	±3.66	±12.11	±0.03	±0.00	±0.34	±0.21	±0.20	±0.04	±6.25	±5.33	±1.14	±0.04	±3.58
Ginger	1000	72.67	62.33	146.33	0.70	0.10	6.43	3.27	3.17	1.00	80.67	60.00	19.67	0.83	75.00
extract		±4.21	±3.25	±12.63	±0.04	±0.00	±0.33	±0.17	±0.18	±0.05	±4.89	±3.52	±1.19	±0.03	±3.96
Recovery	0	84.00	55.00	138.33	0.63	0.13	6.87	3.43	3.44	1.00	85.00	72.00	26.30	0.73	78.00
control		±4.96	±3.54	±12.14	±0.03	±0.01	±0.38	±0.19	±0.18	±0.05	±5.21	±4.69	±1.63	±0.05	±4.21
Recovery	1000	77.33	69.67	136.33	0.71	0.17	7.13	3.46	3.67	0.94	96.67	66.00	22.40	0.73	82.67
high dose		±3.58	±3.66	±12.19	±0.05	±0.02	±0.31	±0.18	±1.17	±0.05	±5.29	±5.61	±1.58	±0.04	±5.64
Ginger ext															

Analysis of variance (ANOVA) was used for statistical analysis. Data are presented as the Mean ± SEM. P>0.05, compared with the respective control. No significant differences were observed between the test and control groups. CHO=Cholesterol; TG=Triglycerides; ALKP=Alkaline phosphatase; T.Bil=Total Bilirubin; D.Bil=Direct Bilirubin; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic pyruvic transaminase; BUN= blood urea nitrogen; FBS=Fasting Blood Sugar

In this study, the repeated administration of ginger extract for 28 days, by oral route, to rats at the highest dosage level of 1000 mg/kg did not induce any observable toxic effects when compared to its corresponding control group of animals. Therefore, this dose may be considered as the No Observed Adverse Effect Level (NOAEL).

TABLE 6: SUMMARY OF HISTOPATHOLOGICAL FINDINGS IN MALE RATS

Organs and observations	Groups	
	Control	Ginger extract High dose
Liver		
Mononuclear cell infiltration	2/3	1/3
Vacuolar changes, hepatocytes	1/3	1/3
Kidneys		
Mononuclear cell infiltration	1/3	0/3
Lungs		
Perivascular mononuclear cell infiltration	2/3	0/3
Peribronchial mononuclear cell infiltration	0/3	1/3
Heart		
Mononuclear cell infiltration	1/3	0/3

All other organs examined were normal

TABLE 7: SUMMARY OF HISTOPATHOLOGICAL FINDINGS IN FEMALE RATS

Organs and observations	Groups	
	Control	Ginger extract High dose
Liver		
Mononuclear cell infiltration	1/3	1/3
Vacuolar changes, hepatocytes	0/3	0/3
Kidneys		
Mononuclear cell infiltration	1/3	0/3
Basophilic tubule	0/3	0/3
Lungs		
Perivascular mononuclear cell infiltration	1/3	1/3
Peribronchial mononuclear cell infiltration	0/3	0/3
Heart		
Mononuclear cell infiltration	1/3	0/3
Pancreas		
Vacuolar change	0/3	0/3
Brain		
Mononuclear cell infiltration	1/3	0/3

All other organs examined were normal

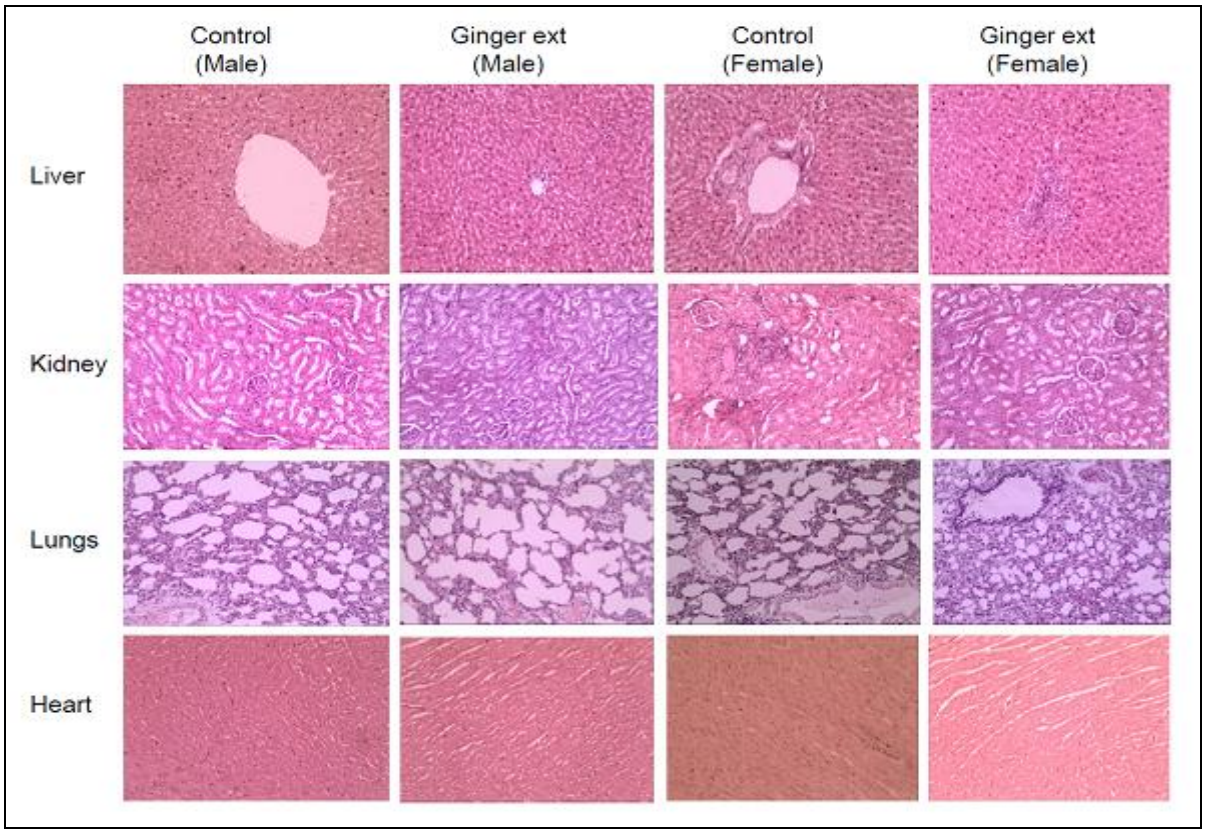

FIG. 3: HISTOPATHOLOGICAL SECTIONS OF LIVER, KIDNEY, LUNGS AND HEART IN 28 DAYS REPEATED DOSE TOXICITY STUDY

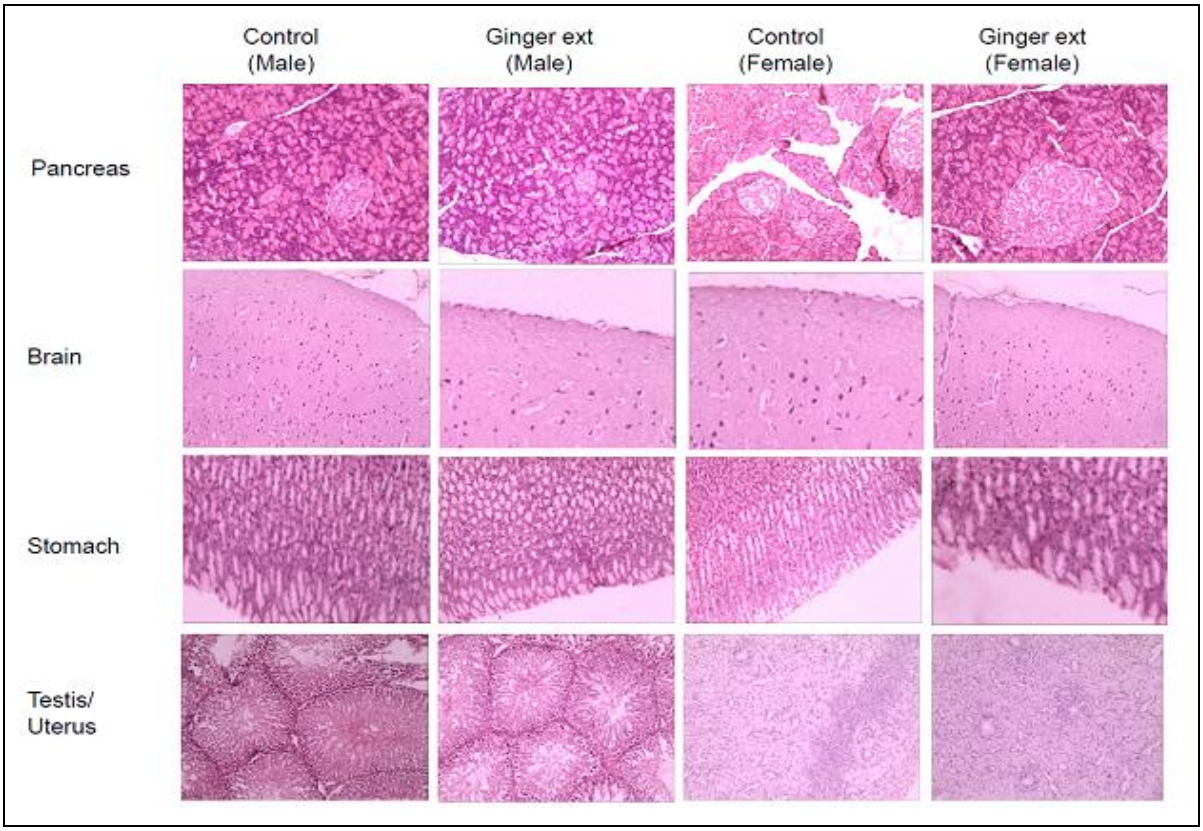

FIG. 4: HISTOPATHOLOGICAL SECTIONS OF PANCREAS, BRAIN, STOMACH AND TESTIS/UTERUS IN 28 DAYS REPEATED DOSE TOXICITY STUDY

DISCUSSION: Ginger is not only an extremely popular dietary condiment used for flavoring food but also an herb that has been used for thousands of years as a medicinal herb to treat a variety of ailments. Chemical and metabolic analyses have revealed that ginger comprises hundreds of compounds and metabolites. The major studied bioactive compounds include gingerols and shogaols, especially 6-gingerol and 6-shogaol, respectively. Research interest in determining the role of natural compounds in preventing disease has increased markedly over the last few years. In spite of the abundance of research studies, many of the results are phenomenon-based and provide data that are descriptive and observational rather than mechanistic. More studies in animals and humans on the pharmacokinetics of ginger and the constituents and on the effects of consumption over a long duration of time are needed.

Although ginger has been used in the clinic for several years, there was still a lack of systemic safety evaluation. In the assessment and evaluation of the toxic characteristics of a test substance, determination of acute oral toxicity study in small animals (mice/rats) is usually an initial step.

Results of acute toxicity obtained in this study clearly indicate that the ginger extract is acutely safe, and the LD_{50} for rats was found to be >2000 mg/kg. The repeated administration of the ginger extract to rats for 28 days demonstrated that oral administration of ginger extract up to 1000 mg/kg to male and female rats was not associated with any abnormalities or mortalities in general conditions, behavior, growth, and food and water consumption of animals. Various parameters of hematology and blood biochemistry were similar in both control and treated animals. The results of necropsy suggest that all of the examined organs treated by 1000 mg/kg of the ginger extract are normal. Converting rat dose to human equivalent dose, the 1000 mg/kg body weight in rats corresponds to about 11 g per day for a human weighing 70 kg [19].

It has been reported that ginger extract interferes with the activities of some digestive enzymes. In diabetic animals which are deficient in the apolipoprotein E gene or have been fed a high-fat diet, ginger significantly lowered serum total cholesterol, LDL, VLDL, and triglycerides and increases the level of HDL [20, 21]. While elucidating the mechanism, it was noted that ginger acts on the

liver to reduce cholesterol biosynthesis and may also stimulate the conversion of cholesterol into bile acids and promote its excretion in the feces [22]. It has also been demonstrated that ginger enhanced the activity of pancreatic lipase and amylase when they were directly in contact with the enzyme [23].

Apart from effects on lipid profile, ginger dose-dependently inhibited the arachidonic acid-induced platelet aggregation, cyclooxygenase-derived thromboxanes and prostaglandins, and prostacyclin synthesis, with a significant increase in fibrinolytic activity, in *in-vitro* and *in-vivo* studies [24-26]. These results indicate the anti-platelet effect of ginger.

Therefore, our concern was whether ginger affects lipid and glucose metabolism and platelets under normal conditions also. In the present toxicity study, treatments of rats with ginger extract up to 1000 mg/kg for 28 days did not affect total cholesterol, glucose level in serum, and triglyceride levels. The platelets count was also normal in all the rats. Our results suggest that ginger extract does not interfere with lipid and glucose metabolism and platelet under normal physiological conditions.

Taken together, the present results from the acute and sub-acute 28 days toxicity study demonstrated no harmful effects of ginger extract in rats.

CONCLUSION: In the acute toxicity study, no toxic signs and symptoms or mortality was observed by feeding ginger extract (containing 8% total gingerols) in any of the animals at the maximum recommended dose level of 2000 mg/kg body weight (LD50 > 2000 mg/kg). In the sub-acute study, the repeated administration of ginger extract for 28 days, by oral route, to rats at the maximum dosage level of 1000 mg/kg did not induce any observable toxic effects when compared to its corresponding control group of animals. Therefore, it may be considered as No Observed Adverse Effect Level (NOAEL).

AUTHOR CONTRIBUTIONS: MB, MRS, and BA were involved in study concept and design; RM and AA were involved in preparation and analysis of extract; NKG and SJ were involved in animal studies and acquisition of data. NKG was involved in the analysis & interpretation of data and drafting of the manuscript. All the authors were involved in revising the manuscript to its final form.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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