Research Article

Safety Assessment of Highly Bioavailable Curcumin Formulation (Curcuwin Ultra+™): Acute and Sub-chronic Oral Toxicity, Mutagenicity, and Genotoxicity Studies

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Abstract

Curcuminoids and their derivatives are known for numerous biological activities including antioxidant, anti-inflammatory, and wound healing properties. However, poor bioavailability of curcuminoids poses significant hurdle in its application as a dietary supplement to improve health conditions. Several commercial preparations have been developed to improve the oral bioavailability of curcumin using formulation technologies. Curcuwin Ultra+™ (CU+) is a water dispersible curcumin formulation that has shown 144 times more bioavailability as compared to standard curcumin in humans. The present study was designed to demonstrate the safety of CU+ through comprehensive list of toxicity studies like acute and sub-chronic oral toxicity, mutagenicity and genotoxicity studies under Good Laboratory Practice (GLP) conditions using OECD guidelines. In an acute oral toxicity study, no lethality was observed in Sprague Dawley rats at dose as high as 2000 mg/kg body weight (b.w.) of CU+ with LD50 cut-off value of 5000 mg/kg b.w. Further, based on the 90-day repeat dose oral toxicity study followed by 28-day recovery period in Sprague Dawley rats, No Observed Adverse Effect Level (NOAEL) was noted to be 1000 mg/kg b.w./day. No mutagenicity due to CU+ was observed with reverse mutation (AMES) test in *Salmonella typhimurium* and *Escherichia coli* strains and no genotoxicity was observed in studies that included micronucleus assay using erythrocytes from Swiss webster mice and, chromosomal aberrations test in Chinese hamster ovary cells. Overall CU+ was found to be safe in rodents, non-mutagenic, non-genotoxic, and had no observed adverse effects under experimental conditions tested.

Keywords: *Curcuma longa*; Turmeric; Curcumin; Curcuminoids; Demethoxycurcumin; Toxicity; Curcuwin Ultra+™

Introduction

Curcuma longa L. (turmeric), is a common spice extensively used in cuisines in India and South Asian countries. In traditional medicine turmeric has been used to treat wide range of disorders including rheumatoid arthritis, osteoarthritis, wound healing, urinary tract infections, and liver ailments. Turmeric is a perennial herb that belongs to Zingiberaceae family containing an active yellow coloured pigment known as curcumin. The turmeric rhizomes contain 75-80% of Curcumin (CUR), 15-20% of Demethoxycurcumin (DMC), 3-5% Bisdemethoxycurcumin (BDMC) [1-5]. Chemical structures of curcumin, demethoxycurcumin, and bisdemethoxycurcumin are provided in Figure 1. Curcuminoids and their derivatives have wide array of biological activities including antioxidant, anti-inflammatory, anticancer, neuroprotective, cardio-protective, radio-protective, antimicrobial and anti-viral properties

[6]. Curcuminoids are known for poor solubility, low absorption and bioavailability, high metabolism rate that restricts its biological applications. Various formulations have been developed to improve oral bioavailability of curcumin followed by validation through human pharmacokinetic studies [7,8].
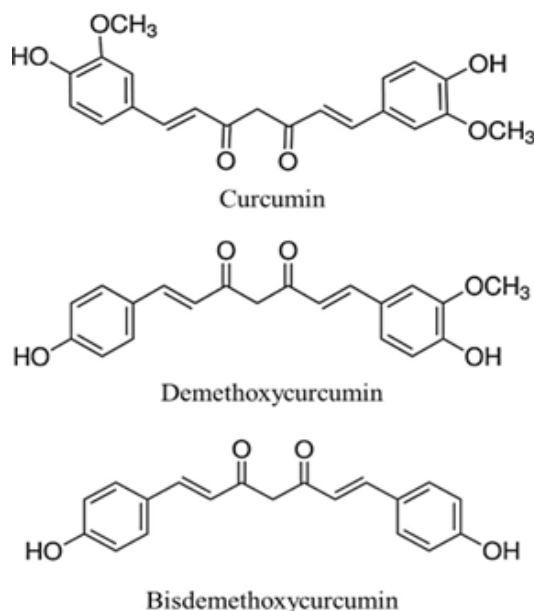
Figure 1: Chemical structure of Curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

CU+ is a novel water-dispersible formulation that is 144 times more bioavailable in humans than standard curcumin [9]. CU+ designed for increased absorption and protection of curcuminoids from alkaline degradation in the intestine, demonstrated superior bioavailability as compared to 95% turmeric extract [10]. CU+ has been found to be safe and well tolerated in clinical studies designed to establish efficacy in human subjects with mild knee osteoarthritis [11] as well as in preclinical experimental studies in rats [12,13]. Further turmeric extract and various curcumin preparations have been found to be safe as established through many experimental and human clinical studies [14-16] and has been approved by the United States Food and Drug Administration as being Generally Recognized As Safe (GRAS) food ingredient [17] and as an approved supplement in several countries [18]. The present study is designed to explore safety of CU+ formulation through single dose acute oral toxicity and 90-day sub-chronic repeated dose oral toxicity studies in Sprague Dawley rats, reverse mutation (AMES) test in *Salmonella typhimurium* and *Escherichia coli* strains, genotoxicity by micronuclei assay done in Swiss webster mice and chromosomal aberrations test in Chinese hamster ovary cells.

Materials and Methods

Test item

Curcuwin Ultra+™, a yellowish orange free flowing homogenous turmeric powder, prepared from rhizomes *Curcuma longa L.*, contained 22.67% of total curcuminoids encapsulated in matrix of cellulose polymer, solubilizer, acidifier and antioxidant manufactured by OmniActive Health Technologies Limited, India.

Acute oral toxicity

The acute oral toxicity study was performed according to Organization for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals (No. 423, Section 4, Health Effects) using female Sprague Dawley strain rats from Vivo Bio Tech Limited, (Siddipet, India). The study was performed in laboratory approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) under registration number 1117/PO/RcBiNRc-L/07/CPCSEA following all ethical practices as laid down in the guidelines for animal care. In two separate steps, three female rats each (8-9 weeks of age) were administered with a single dose of 2000mg/kg b.w. of CU+ in corn oil, using oral gavage for Step I and Step II experiments. As all the three rats dosed in Step I survived, three additional rats were given a dose of 2000mg/kg b.w. (Step II). In both the steps, animals were observed at 1-, 2- and 4-hours post dosing and from day 2 onwards observed once daily for 14 days for any clinical signs of morbidity or mortality. On Day 15, all the animals were euthanized for gross pathological examinations, clinical signs, and body weight. Mean and standard deviation of the results were calculated using SYSTAT Software Version 13.

Sub-chronic oral toxicity

Study design: The sub-chronic oral toxicity study was conducted using OECD guidelines for testing chemicals, health effects test guidelines, for repeated dose 90-day oral toxicity study in rodents, section 408 and US FDA Redbook 2000: IV.C.4.a Sub-chronic Toxicity Studies with Rodents using OECD Good Laboratory Practices (GLP). The study was performed in a Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) approved laboratory under registration number 1117/PO/RcBiNRc-L/07/CPCSEA following all ethical practices as laid down in the guidelines for animal care.

Animals: Sprague dawley strain rats (50 males and 50 females), 6 weeks old from Vivo Bio Tech Limited (Siddipet, India) were used for the study after physical examination. Female rats used in the study were nulliparous and non-pregnant. The animals were housed according to standard guidelines in groups of three in standard polypropylene cages with autoclaved clean corn cob bedding. The animal room was air-conditioned with adequate air changes per hour (at least 20 fresh air changes) and a 12h light/dark cycle. The experimental room was continuously monitored for temperature and relative humidity. The room temperature and relative humidity were 20 °C to 23 °C and 45 to 65%, respectively. The animals were allowed to acclimatize for a minimum of five to six days before the initiation of experiments. Special Diets Services (SDS) pellet feed (England) and drinking water was provided *ad libitum* throughout the study period.

Treatment: Sprague Dawley rats were divided into six groups (four main groups - 10/sex/group and two recovery groups - 5/

sex/group) using stratified randomization for body weight. At randomization, the animals were approximately 6-7 weeks old, and their body weight was within ±20% of the overall mean of each sex. Curcuwin Ultra+™ was administered to rats through oral gavage once daily at a dose of 250 (Group II – low dose), 500 (Group III – mid dose), or 1000 (Group IV-high dose) mg/kg b.w. at dose volume of 10mL/kg in corn oil for 90 consecutive days. Group I served as a vehicle control with no dosage of CU+ in corn oil. Two additional groups of animals for the recovery study received 0 (Group V-R) and 1000 (Group VI-R) mg/kg b.w./day of CU+ in corn oil for 90 days, followed by no additional treatment for 28 days. The analytical verification of test article was carried out for CU+ using HPLC method. The product has been confirmed to be stable for two years. The dose formulations were prepared by thoroughly mixing appropriate amounts of CU+ in corn oil shortly before dosing and homogeneity of test article was maintained during administration period using a magnetic stirrer. During the course of the sub-chronic study, all animals were provided *ad libitum* feed, until the day prior to the scheduled euthanasia. At completion of the 90-day dosing period, all animals in main groups were euthanized. In the recovery group, after completion of the 90-day treatment period, the animals were kept under post treatment observation for 28 days and then euthanized.

Parameters investigated:

Clinical signs, body weight and feed consumption: All animals were observed twice daily for mortality and morbidity during working days and once during holidays. Clinical signs were recorded once daily during the treatment period. Clinical observations included changes in handling response, skin, fur, eyes and mucous membranes, breathing pattern, presence of secretions and excretions; ears, eyes, oral cavity, ventral and posterior side of the body, presence of injury, wound or swelling, posture, gait and behaviour. Ophthalmological examination was performed using a Welch Allyn direct ophthalmoscope. The weight of each rat was recorded at day 1 and weekly intervals throughout the study period. Mean body weights and mean body weight changes were calculated for the corresponding intervals. The amount of feed consumed by animals in each cage was recorded weekly. Feed intake was calculated as gram/animal/day for the corresponding body weight intervals.

Clinical pathology: Urine and blood samples were collected for clinical evaluations (urinalysis, haematology, serum chemistry, and thyroid hormones) from all animals of main group at end of treatment period on 91-day and from animals of recovery groups on day 119, prior to the scheduled necropsy. For urine collection, each animal was kept in a metabolic cage in fasting for 12-14h and the urine sample was collected into a specimen vial. Urinalysis included volume, appearance, specific gravity, color, pH, blood cells, bilirubin, urobilinogen, leucocytes, ketone bodies, proteins, glucose and nitrite. Additionally microscopic examination of urine was also carried out. Urine analysis was performed using Siemens Cliniteck status urine analyzer with Multistix® 10 SG urine analysis Strips. Blood samples were collected from all animals under light isoflurane anaesthesia.

Before blood collection the animals were fasted overnight. During the fasting period, animals were allowed access to water *ad libitum*. Blood samples were drawn from the retro-orbital plexus using a micro-hematocrit heparinized glass capillary tube. Blood samples were centrifuged, and the plasma was separated for clinical biochemistry analysis. Hematology parameters were analyzed using Siemens ADVIA 2012i Hematology Analyzer and included: White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelet Count (PLT), Mean Platelet Volume (MPV), Differential Leukocyte Count (DC)-neutrophils, lymphocytes, monocytes, eosinophils, and basophils and reticulocyte count (Retic) and Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) were measured using Sysmex CA-620 coagulation analyzer. Clinical biochemistry parameters were analyzed using Siemens Dimension ExL 200 clinical chemistry analyzer and included: albumin, globulin, A/G ratio, glucose, Blood Urea Nitrogen (BUN), creatinine, total cholesterol, triglycerides, low-density lipoprotein cholesterol (ALDL), High-Density Lipoprotein Cholesterol (AHDL), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transpeptidase (GGT), total bilirubin, Calcium (Ca), phosphorus, Total Protein (TP). Electrolytes Sodium (Na), Potassium (K), Chloride (Cl), were analysed using Easy Lyte Na+/K+/Cl- analyzer. Thyroid hormones Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH) were estimated using ELISA method.

Necropsy, organ weight and histopathology: On the completion of treatment period, a complete necropsy was performed on all animals. Animals were euthanized under carbon dioxide asphyxiation. At the scheduled necropsies (on Day 91 for main group I–IV and Day 119 for recovery group) the following organs were weighed from all animals: brain, thymus, spleen, ovaries, heart, kidneys, testes, liver, adrenals, pituitary and epididymis, prostate + seminal vesicles with coagulation glands. Over 38 tissues and organs (except eyes, testes, and epididymis) were collected and placed in 10% neutral-buffered formalin for microscopic examinations. For histopathology, tissues were processed, embedded in paraffin blocks, sectioned at 4 to 5 micrometres thickness and stained with haematoxylin and eosin.

Statistical analysis

Statistical analysis was performed using SYSTAT Version 13. Body weight, feed consumption, organ weights, clinical pathology, haematology, clinical chemistry, urine analysis, thyroid estimation data were analysed using statistical methods. All the data were checked for normality with Shapiro–Wilk's test, and homogeneity of variance by Barlett's Test. Data for each group of animals were subjected to analysis of variance (ANOVA). Values were given as mean±Standard Deviation (SD). Dunnet's t-test was used to compare the difference between treated and control groups. Statistical significances at a 5% ($p < 0.05$) level of significance were used in the analyses.

Mutagenicity study

Mutagenic activity of CU+ was investigated using AMES test in compliance with GLP requirements based on OECD Principles of GLP as revised in 1997. The study was conducted as per study plan outlined prior to execution of the test and the OECD Guidelines 471. The mutagenicity study was conducted at Vivo Bio tech Technologies Ltd. (Siddipet, India). The tester strains used in this study were histidine-requiring auxotrophic strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537) and *E. coli* (WP2 uvrA (pKM101)) obtained from molecular toxicology Inc., 157 industrial park dr. boone, NC 28607, US. Strains TA100 and TA1535 are constructed to detect base-pair substitutions, while strains TA98 and TA1537 allow detection of mutagens that cause frameshift mutations. Metabolic activation was performed using a cofactor-supplemented post-mitochondrial fraction (S9 fraction). Curcuwin Ultra+™ was dissolved in dimethyl sulfoxide (DMSO) and was tested in two independent experiments at a concentration of 1600, 800, 400, 200,100μg/plate. The strain-specific positive control; 4-nitro-1,2-phenylenediamine at 10μg/plate for *S. typhimurium* strain TA98 and 50μg/plate for TA 1537, sodium azide at 10μg/plate for strains TA100 and TA1535, and methyl methane sulfonate at 208μg/plate for strain *E. Coli* (WP2 uvrA (pKM101)) were also used. For conditions of metabolic activation, 2-aminoanthracene at 2.5μg/plate [10.0μg/plate in *E. coli* (WP2 uvrA (pKM101))] for all *S. typhimurium* strains was used as a positive control. Vehicle control and sterile RO water were used as a negative control.

During the experiments, an overnight culture of the test strain in nutrient broth and molten agar, along with S9 mix/S9 mix substitution buffer was mixed either with appropriately diluted CU+ concentrate or negative or vehicle or positive controls. In the pre-incubation assay, 100μL test solution, 500μL S9 mix/S9 mix substitution buffer and 100μL bacterial suspensions were mixed in a test tube and incubated at 37±2 °C for approximately 21-30min. After pre-incubation 2.0ml overlay agar (45±2 °C) was added to each tube. The mixture was poured on minimal agar plates. After solidification the plates were incubated upside down approximately for 67h 20min in Phase I and 68h 55min in Phase II at 37±2 °C in the dark. Curcuwin Ultra+™ at different concentrations and the controls (vehicle control, negative controls, and positive controls) were tested in triplicate. The colonies were counted manually. The mean values from the plates for each concentration together with standard deviations were compared to the spontaneous reversion rates.

Micronucleus study

The in vivo micronucleus test evaluated the genotoxic potential of CU+ to induce the formation of micronuclei in polychromatic erythrocytes in the bone marrow of Swiss Webster mice. The study was conducted in compliance with OECD 474 (2016)24 and GLP C (97)186/Final. On the day of treatment, the test article CU+ was suspended in corn oil at 200mg/mL to achieve concentrations of 0, 500, 1000, and 2000mg/kg b.w./day and was administered at a constant dose volume of 10mL/kg. Forty (40) mg of freshly prepared cyclophosphamide monohydrate in deionized water at a concentration of 4mg/mL served as the positive control and corn oil served as the vehicle control. Swiss Webster mice, 6-8-week-old both male and female used for the study were acclimatized, observed, and examined for a period of 5 days to confirm that the animals were in good health. Animals were housed individually in sterilized polycarbonate cages with autoclaved clean corn cob bedding material; the room temperature was 20.3-23.2 °C with 45.0-63.0% relative humidity and 12-hour light-dark cycles. All animals had access to standard pellets (SDS, England) and water *ad libitum*. Animals were randomized into groups of 5 animals/sex/group for study groups including negative and positive control groups.

The test article (CU+ at dose of 500, 1000 and 2000mg/kg b.w./day) and vehicle control were administered twice at an interval of approx. 24h by oral gavage using 1mL disposable syringe. The positive control was administered on Day 2. The volume of dose administered to each animal was calculated based on the most recent body weight. Animals were observed for clinical signs at pre-dose, and at 1, 2, 3, 4 and 24h post-dosing. Positive control animals were dosed once on day 2 and observed twice daily for mortality or moribund condition. Body weights were recorded on the day of receipt of animals, before randomization, on treatment days, and the day of sacrifice for both the phases of the study. Bone marrow samples were collected from both exposed femurs of each animal at 18 to 24 hours after the last dose. Slides were prepared, blind coded, and examined for incidence of micronucleated cells. A minimum of 4000 Polychromatic Erythrocytes (PCEs) were analysed per animal and the frequency of micronucleated PCEs was reported as percentage. The proportion of polychromatic erythrocytes of total erythrocytes was determined for each animal by counting a total of at least 500 erythrocytes.

Chromosomal aberration study

The chromosomal aberration test is an *in vitro* genotoxicity test that is conducted using a mammalian cell line. The study was conducted in two phases-pre-experiment for cytotoxicity and main study with CU+ exposure: short term (for 3 hours; with or without metabolic activation system) and long term (24 hours exposure; without metabolic activation system). The study was conducted as per OECD guidelines TG 473(4) 2016: "*In vitro* mammalian chromosomal aberration test" and GLP (1997). The Chinese hamster ovary K1 (CHO-K1) cells were procured from American Type Culture Collection (ATCC) and ensured that it is free from mycoplasma contamination. The cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% antibiotics and 1% Sodium Pyruvate at 37±2 °C in a 5% CO_2 humidified incubator. CHO-K1 cells were seeded in 25cm² flasks at a density of 2.5×10^5 cells/mL culture medium and incubated for approximately 23-24h.

For cytotoxicity test, 100mg CU+ was diluted in 2mL DMSO at a concentration of 50mg/mL. For main study, 250mg of test item was diluted in 20mL DMSO to obtain a final concentration of 12.5mg/

mL. Further dilutions were made from the above stock concentrates and used in the experiments. The dose formulation analysis was performed using a validated HPLC method. The cells were exposed to test formulations with and without S9 for 3 hours; without S9 for 24 hours and counted under Haemocytometer for determining cytotoxicity. The main study was performed for each concentration of the test item along with vehicle, negative and positive controls in duplicates. Two hours prior to harvesting, colchicine solution at a final concentration of 0.3 μg/mL was added to each culture and the cells were fixed, stained with 5% freshly prepared Giemsa stain and analysed for chromosomal aberrations such as chromatid breaks, gap, chromatid and chromosome type aberration. The results of the chromosomal aberration test were analysed by Fisher's Exact Test with a 5% significance level using Graph Pad Prism statistical software version 7.01.

Results

Acute oral toxicity study

No clinical signs of toxicity were observed in animals after CU+ administration up to day 15 in step I and II of the experiment.

No abnormality was detected in animals during gross pathology observations. Based on the results, LD50 of CU+ was reported to be greater than 2000-5000mg/kg b.w. and LD50 cut-off value was 5000mg/kg b.w. (category 5 or unclassified).

Sub-chronic oral toxicity study

Clinical signs, body weight and feed consumption: No test item related mortality and clinical signs were observed in any of the treated groups during the experimental period. Detailed clinical examination showed no test item related clinical signs across all the study groups in both sexes. No statistically significant difference in the body weight and its gain was observed in any of group and between both sexes except for a significant decrease in body weight gain in high dose recovery group-1000mg/kg/b.w./day (Figures 2 & 3) which is considered as incidental with no correlation with feed consumption. Further, a reduction in body weight of animals were noted on day 91 and 119 attributed to fasting. No statistically significant difference in the feed consumption was observed in 250, 500 and 1000 mg/kg/b.w./day group animals when compared with vehicle in main and recovery groups in both sexes.
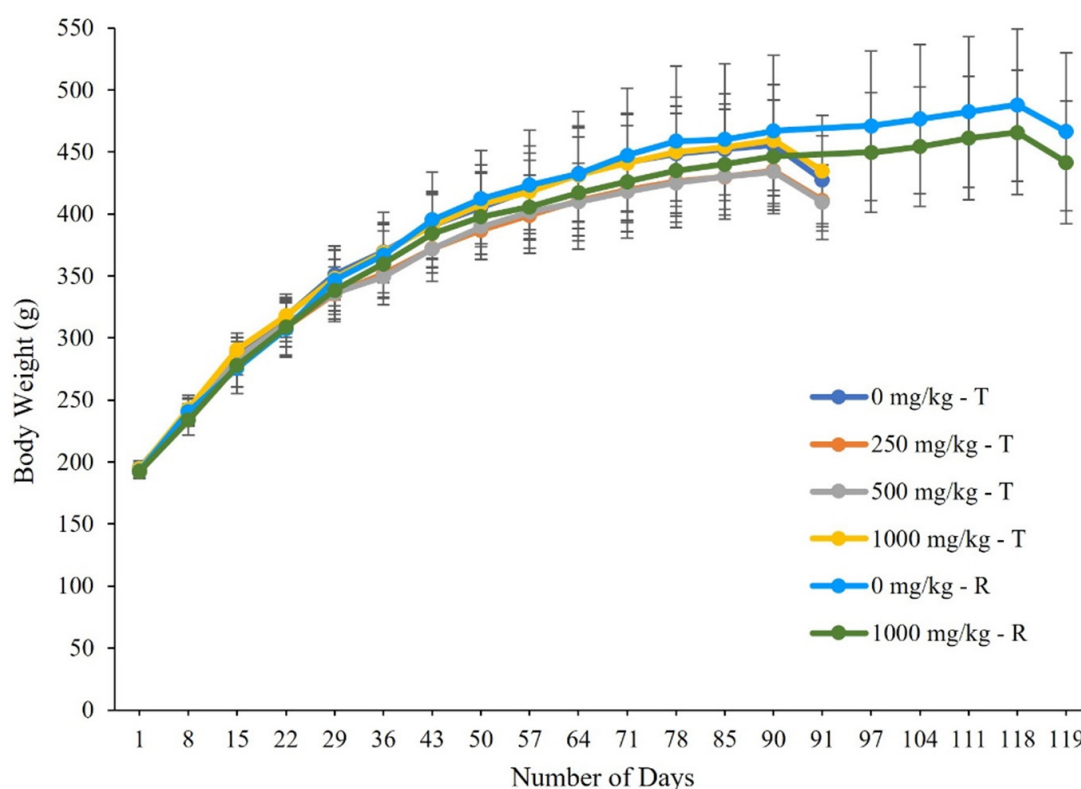
Figure 2: Effect of CU+ concentrate on body weights in male rats. Mean body weights for male rats during a 90-day oral (gavage) toxicity study and 28-day recovery study with CU+ concentrate. The values are presented as means±standard deviation [10 rats/sex/group for test (T) groups and 5 rats/sex/group for recovery (R) group].
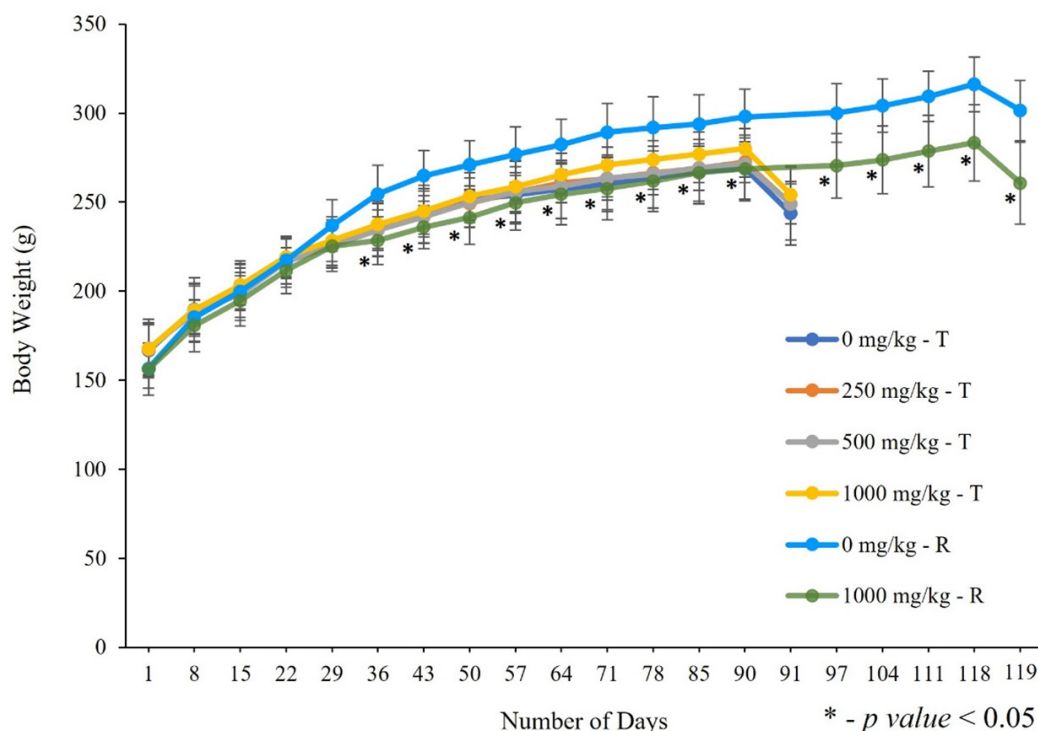
Figure 3: Effect of CU+ concentrate on body weights in female rats. Mean body weights for male rats during a 90-day oral (gavage) toxicity study and 28-day recovery study with CU+ concentrate. The values are presented as means±standard deviation [10 rats/sex/group for test (T) groups and 5 rats/sex/group for recovery (R) group]. * - indicates 0mg/kg–R group is statistically significant different at p<0.05 when compared with 1000mg/kg–R group.

Clinical pathology

Urinalysis: No statistically significant difference was observed in the urine analysis across groups.

Haematology: No significant difference in the haematology was observed in group 250, 500 and 1000mg/kg/b.w./day when compared with vehicle group (Tables 1 & 2). In recovery group, 1000mg/kg/b.w./day males showed statistically significant increase in absolute lymphocytes compared to vehicle group. The changes were considered to be spurious as there was no increase of absolute lymphocytes in main groups. The change is considered as biological variation and not having any toxicological significance. No morphological abnormality was detected in blood smear across all groups. No cellular and morphological abnormality detected in bone marrow smear examination across all groups.

Table 1: Effect of CU+ concentrate on hematological parameters in male rats.

Group			G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=5)	G6 (n=5)
Dose (mg/kg b.w./day)			0	250	500	1000	0	1000
	Parameters	Units	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Parameters	WBC	x10^3 cells/µL	7.45±1.22	7.83±1.64	7.55±1.57	8.05±1.14	7.38±1.52	8.76±0.91
	RBC	x10^6 cells/µL	9.19±0.3	9.02±0.29	9.39±0.4	9.33±0.34	9.2±0.44	9.28±0.52
	HGB	g/dL	16.46±0.47	16.02±0.42	16.42±0.67	16.49±0.36	16.18±0.74	15.86±0.86
	HCT	%	45.52±1.02	44.58±1.17	45.38±1.8	45.62±1.09	43.76±1.75	43.8±1.27
	MCV	fL	49.53±1.26	49.43±1.69	48.39±1.32	48.93±1.12	47.64±1.78	47.28±2.21
	MCH	pg	17.91±0.58	17.79±0.64	17.5±0.42	17.69±0.45	17.58±0.75	17.14±1.1
	MCHC	g/dL	36.14±0.38	35.96±0.32	36.17±0.7	36.14±0.45	36.98±0.73	36.2±1.06
	RDW	%	12.13±0.34	12.19±0.43	12.06±0.39	12.28±0.35	12.38±0.48	12.38±0.54
	PLT	x10^3 cells/µL	952.8±86.01	931±118.19	949.3±182.4	923±123.84	811.4±125.92	973.2±134.76
	MPV	fL	6.7±0.41	6.57±0.44	6.58±0.49	6.83±0.41	7.6±0.19	7.5±0.25
	RRC	%	1.53±0.2	1.49±0.24	1.42±0.29	1.41±0.15	1.6±0.17	1.73±0.2
	ARC	x10^9 cells/L	140.55±19.24	134.4±21.15	132.86±23.35	131.68±14.09	146.74±11.66	160.22±22.96
	PT	Sec	8.84±0.83	8.53±0.92	8.67±0.81	8.79±0.76	10.6±1.43	9.92±1.43
	APTT	Sec	19.96±1.59	19.73±1.53	18.73±1.31	18.79±1.73	21.02±1.04	20.72±1.37
Relative Differential Leukocyte Count	Neut	%	16.57±4.17	17.81±6.33	17.79±4.67	18.49±8.63	18.52±4.87	14.3±3.44
	Lymph	%	74.16±5.68	73.18±6.98	72.27±6.77	72.33±9.14	73.92±5.26	78.02±4.6
	Mono	%	5.15±1.59	4.92±1.61	5.46±1.86	4.95±1.6	4.02±1.24	4.16±1.28
	Eos	%	1.96±0.42	1.75±0.6	2.15±0.85	1.88±0.44	2.22±0.89	2.04±0.72
	Baso	%	0.19±0.09	0.13±0.05	0.16±0.08	0.18±0.11	0.08±0.04	0.08±0.04
Absolute Differential Leucocyte Count	Neut	x10^3 cells/µL	1.23±0.3	1.44±0.81	1.32±0.32	1.46±0.64	1.41±0.64	1.26±0.39
	Lymph	x10^3 cells/µL	5.54±1.03	5.66±0.92	5.5±1.5	5.86±1.27	5.42±0.94	6.82±0.63*
	Mono	x10^3 cells/µL	0.38±0.14	0.4±0.19	0.4±0.11	0.4±0.14	0.29±0.08	0.37±0.13
	Eos	x10^3 cells/µL	0.15±0.04	0.14±0.06	0.16±0.07	0.15±0.04	0.17±0.08	0.18±0.07
	Baso	x10^3 cells/µL	0.01±0.01	0.01±0	0.01±0.01	0.02±0.01	0.01±0	0.01±0.01

*- G6 indicates statistically significant changes at $p<0.05$ when compared with G5. WBC - White Blood Cell, RBC-Red Blood Cell, HGB-Haemoglobin Concentration, HCT–Haematocrit, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin, MCHC-Mean Corpuscular Haemoglobin Concentration, RDW-Red Cell Distribution Width, PLT-Platelet Count, MPV-Mean Platelet Volume, RRC–Relative Reticulocyte Count, ARC-Absolute Reticulocyte Count, PT-Prothrombin Time, APTT -Activated Partial Thromboplastin Time, Neut–Neutrophils, Lymph–Lymphocytes, Mono–Monocytes, Eos–Eosinophils, Baso–Basophils.

Table 2: Effect of CU+ concentrate on hematological parameters in female rats.

Group			G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=5)	G6 (n=5)
Dose (mg/kg b.w./day)			0	250	500	1000	0	1000
	Parameters	Units	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
	WBC	x10^3 cells/µL	6.58±1.38	6.32±2.2	6.35±1.33	5.64±1.47	5.5±1.37	4.59±1.23
	RBC	x10^6 cells/µL	8.71±0.27	8.47±0.32	8.57±0.39	8.23±0.65	8.44±0.21	8.42±0.25
	HGB	g/dL	16.23±0.43	15.66±0.54	15.99±0.32	15.61±0.82	15.96±0.34	15.7±0.37
	HCT	%	43.95±1.19	42.54±1.23	43.39±1.06	42.6±1.88	42.14±0.93	41.74±1.07
	MCV	fL	50.48±1.41	50.23±1.19	50.66±1.13	51.96±2.4	49.92±0.9	49.58±1.3
	MCH	pg	18.62±0.63	18.51±0.77	18.66±0.52	20.82±5.77	18.92±0.38	18.64±0.5
Parameters	MCHC	g/dL	36.91±0.52	36.83±0.82	36.84±0.36	36.73±0.85	37.86±0.39	37.64±0.7
	RDW	%	11.12±0.4	11.47±0.61	11.02±0.28	11.32±0.65	11.5±0.19	11.28±0.41
	PLT	x10^3 cells/µL	899.8±209.88	873.4±93.89	951.4±141.13	928.9±66.34	832±115.08	890.8±80.11
	MPV	fL	6.84±0.27	6.86±0.53	6.63±0.36	6.43±0.3	7.02±0.57	6.92±0.41
	RRC	%	1.49±0.28	1.71±0.29	1.76±0.47	1.79±0.48	1.74±0.19	1.58±0.49
	ARC	x10^9 cells/L	129.36±22.68	145.05±24.67	150.78±40.53	144.8±32.85	147.26±15.31	133.38±41.79
	PT	Sec	6.93±0.74	7.68±1.22	7.03±0.87	7.98±2.45	11.28±1.51	11.28±1.49
	APTT	Sec	16.88±2.1	15.51±2.27	15.77±2.31	15.49±2.21	19.94±1.15	20.22±0.86
	Neut	%	18.7±14.78	17.6±7.6	22.3±5.94	19.79±7.79	21.58±9.47	29.48±24.02
Relative Differential Leukocyte Count	Lymph	%	73.03±15.85	75.02±8.15	68.69±6.21	71.12±8.19	68.14±12.34	60.3±28.04
	Mono	%	4.01±1.25	3.81±1.31	4.71±0.91	4.61±1.35	5.3±2.51	6.66±5.96
	Eos	%	1.88±0.73	1.7±0.69	1.79±0.67	2±0.71	3.2±1.02	2.24±1.33
	Baso	%	0.13±0.05	0.09±0.06	0.11±0.06	0.08±0.06	0.08±0.04	0.06±0.05
	Neut	x10^3 cells/µL	1.15±0.76	1.11±0.6	1.41±0.48	1.06±0.4	1.11±0.33	1.53±1.69
Absolute Differential Leucocyte Count	Lymph	x10^3 cells/µL	4.88±1.63	4.72±1.79	4.37±1.13	4.06±1.3	3.84±1.35	2.55±1.18
	Mono	x10^3 cells/µL	0.26±0.09	0.25±0.13	0.29±0.06	0.27±0.1	0.27±0.08	0.35±0.42
	Eos	x10^3 cells/µL	0.13±0.05	0.11±0.06	0.11±0.04	0.11±0.04	0.17±0.05	0.09±0.07
	Baso	x10^3 cells/µL	0.01±0.01	0.01±0.01	0.01±0	0.01±0.01	0±0	0±0.01

Clinical biochemistry: Statistically significant increase was observed in albumin, calcium, total protein, globulin in 1000mg/kg/b.w./day main group and phosphorus in 500 and 1000mg/kg/b.w./day main group males; ALDL in 250 and 1000mg/kg/b.w./day main group female rats when compared to vehicle group (Tables 3 & 4). In recovery group, 1000mg/kg/b.w./day dosed group females showed increased phosphorus and decreased chloride, and males showed decreased potassium. The above changes did not correlate with histopathology findings and the mean values are within the reference range for the laboratory. Hence, these were considered as incidental and not having any toxicological significance.

Table 3: Effect of CU+ concentrate on blood biochemistry parameters in male rats.

Group			G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=5)	G6 (n=5)
Dose (mg/kg b.w./day)			0	250	500	1000	0	1000
	Parameters	Units	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Parameters	XALB	g/dL	3.44±0.17	3.48±0.22	3.54±0.13	3.66±0.14*	3.56±0.13	3.72±0.23
	ALPI	U/L	101.1±15.02	99.7±19.49	110.4±29.33	103.5±21.16	82±11.22	85.4±15.24
	ALTI	U/L	49.8±17.76	36.1±9.22	37.1±7.26	40.1±11.54	50±24.42	54.4±35.8
	AST	U/L	122.9±35.33	109.8±22.26	98.1±13.98	107.3±23.21	109.4±28.88	110.6±48.08
	BUN	mg/dL	19.1±1.85	18±1.94	18.9±1.85	20.7±2	20.4±1.67	17.6±2.88
	CA	mg/dL	10.24±0.27	10.43±0.22	10.44±0.21	10.57±0.28*	10.9±0.25	10.96±0.49
	TBI	mg/dL	0.07±0.02	0.06±0.03	0.08±0.02	0.08±0.03	0.05±0.02	0.05±0.04
	TGL	mg/dL	56.6±30.67	38.4±8.49	73±100.53	53.2±17.54	61.8±17.68	102.6±48.72
	CHOL	mg/dL	88.5±7.52	81.9±8.4	85.8±12.21	89.6±7.15	72.4±7.96	67±12.21
	ALDL	mg/dL	23.3±1.83	23.9±1.85	25.3±2.11	23.7±2.21	20.6±3.21	18.6±1.82
	AHDL	mg/dL	75.9±6.14	71.6±6.17	74.2±11.94	78.8±6.41	67.4±6.19	65±7.62
	GLUC	mg/dL	126.9±18.65	118.9±22.73	117.7±19.94	123.6±19.41	133.6±14.6	138±39.83
	TP	g/dL	6.85±0.39	6.87±0.36	7.18±0.19	7.40±0.37*	7.12±0.23	7.26±0.3
	GLOB	g/dL	3.41±0.25	3.39±0.17	3.64±0.13	3.74±0.28*	3.56±0.17	3.54±0.4
	A/G Ratio	-	1.01±0.05	1.03±0.05	0.97±0.04	0.98±0.06	1±0.06	1.07±0.19
	CRE2	mg/dL	0.46±0.06	0.46±0.06	0.5±0.07	0.49±0.06	0.5±0.06	0.47±0.07
	PHOS	mg/dL	4.87±0.47	5.46±0.39	5.51±0.64*	5.67±0.71*	4.68±0.54	4.82±0.26
	Na+	mmol/L	141.07±0.87	141.18±0.96	141.3±0.96	141.13±0.38	141.44±0.17	141.3±0.19
	K+	mmol/L	4.35±0.25	4.25±0.26	4.17±0.33	4.42±0.2	4.44±0.14	4.17±0.18**
	Cl⁻	mmol/L	104.45±2	104.19±1.32	104.3±1.45	104.04±0.85	104.78±1.14	103.6±1.39
	Urea	mg/dL	40.87±3.97	38.52±4.16	40.45±3.97	44.3±4.29	43.66±3.58	37.66±6.17
	GGT	U/L	7.3±1.06	6.8±0.92	6.7±0.82	6.9±0.88	7.2±0.84	7.2±0.84

*-indicates statistically significant changes at p<0.05 when compared with G1; **-indicates statistically significant changes at p<0.05 when compared with G6. XALB -Albumin, ALPI-Alkaline Phosphatase, ALTI-Alanine aminotransferase, AST-Aspartate aminotransferase, BUN - Blood Urea Nitrogen, CA–Calcium, TBI-Total Bilirubin, TGL–Triglyceride, CHOL-Total Cholesterol, ALDL-Low-Density Lipoprotein Cholesterol, AHDL - High-Density Lipoprotein Cholesterol, GLUC–Glucose, TP-Total Protein, GLOB–Globulin, A/G Ratio–Albumin Globulin Ratio, CRE2–Creatinine, PHOS–Phosphorus, Na+-Sodium, K+-Potassium, Cl--Chloride, GGT-Gamma glutamyl transpeptidase.

Table 4: Effect of CU+ concentrate on blood biochemistry parameters in female rats.

Group			G1 (n=10)	G2 (n=10)	G3 (n=10)	G4 (n=10)	G5 (n=5)	G6 (n=5)
Dose (mg/kg b.w./day)			0	250	500	1000	0	1000
	Parameters	Units	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Parameters	XALB	g/dL	3.59±0.22	3.48±0.17	3.61±0.16	3.7±0.16	3.57±0.18	3.53±0.19
	ALPI	U/L	67.6±25.28	57.6±10.66	64.5±17.91	62.6±27.75	45.4±2.61	59±20.26
	ALTI	U/L	27.6±9.54	27.8±12.02	36.1±27.64	25.8±8.11	20.2±7.19	28.8±10.99
	AST	U/L	101.9±17.32	115.7±36.2	116.7±54.53	101.4±14.42	88±8.51	103.2±23.54
	BUN	mg/dL	21.2±2.1	22.6±3.27	21.5±2.59	23.6±2.55	19.4±1.67	23.2±3.42
	CA	mg/dL	10.29±0.27	10.28±0.29	10.46±0.32	10.39±0.22	10.6±0.14	10.74±0.5
	TBI	mg/dL	0.04±0.02	0.04±0.02	0.06±0.03	0.06±0.04	0.05±0.02	0.04±0.03
	TGL	mg/dL	34.3±15.92	26.9±5.43	27.6±8	34.5±20.75	43.8±23.95	26.2±4.49
	CHOL	mg/dL	73.3±8.72	79.2±9.4	80.8±14.75	82±9.45	73.6±9.45	70±5.57
	ALDL	mg/dL	17.8±1.4	19.60±1.58*	18.7±1.57	19.80±1.62*	16.8±0.45	17±1
	AHDL	mg/dL	69.3±6.6	73.6±8.33	75.7±10.79	75.5±7.72	66.6±5.68	66.2±4.66
	GLUC	mg/dL	117±11.32	129±27.1	141.2±29.23	139.7±24.45	133.2±14.7	135±28.78
	TP	g/dL	6.91±0.31	6.82±0.3	7.04±0.24	7.17±0.31	7.04±0.23	6.72±0.49
	GLOB	g/dL	3.32±0.12	3.34±0.17	3.43±0.21	3.47±0.18	3.47±0.15	3.19±0.34
	A/G Ratio	-	1.09±0.06	1.04±0.05	1.05±0.08	1.07±0.04	1.03±0.07	1.12±0.09
	CRE2	mg/dL	0.52±0.07	0.53±0.07	0.54±0.08	0.56±0.05	0.54±0.04	0.54±0.09
	PHOS	mg/dL	4.55±0.76	4.55±0.54	4.72±0.81	4.92±0.47	4.34±0.29	5.06±0.51**
	Na+	mmol/L	139.31±1.08	139.22±1.04	139.65±1.28	139.05±1.26	139.6±0.79	138.7±0.32
	K+	mmol/L	4.05±0.3	3.93±0.15	3.83±0.28	3.91±0.3	3.83±0.22	4.08±0.23
	Cl⁻	mmol/L	104.4±2.45	104.01±2.01	105.3±2.25	104.64±2.02	106.02±1.13	104.06±1.15**
	Urea	mg/dL	45.37±4.49	48.36±7	46.01±5.55	50.5±5.45	41.52±3.58	49.65±7.32
	GGT	U/L	6.5±0.85	6.9±0.88	6.8±1.4	6.2±0.79	5.8±0.45	6±0.71
	GGT	U/L	6.5±0.85	6.9±0.88	6.8±1.4	6.2±0.79	5.8±0.45	6±0.71

*-indicates statistically significant changes at p<0.05 when compared with G1; ** - indicates statistically significant changes at p<0.05 when compared with G6.

Necropsy, organ weight and histopathology

No treatment-related abnormality was observed in gross pathology in any animals across all group and sexes. However, hydronephrosis was observed in one male animal in 1000mg/kg/b.w./day main group. Distended uterus with watery content was observed in four females of vehicle control, three female animals of low dose and high dose groups and two female animals of mid dose on day 91. Also, the later condition was observed in one female rat of high dose recovery group on day 119. The changes observed in kidney was considered to be incidental finding and the change observed in uterus was physiological and considered as incidental.

Organ weight: No statistically significant difference was observed in absolute organ weights except a significant decrease in absolute kidney, absolute spleen weight in 500mg/kg/b.w./day main group males, significant increase in absolute liver weights in 500 and 1000mg/kg/b.w./ day main group females, absolute lungs weight in 1000 mg/kg/b.w./day main group females and absolute pituitary weight in 500mg/kg/b.w./day main group

females compared to vehicle group. Also, there was a significant decrease in absolute prostate, seminal vesicles, coagulation gland weights taken after fixation of tissues in in males' of 1000 mg/kg/b.w./day compared to vehicle group at the end of recovery period. No significant difference in relative organ weights were observed in any groups as compared to vehicle group except for a significant increase in relative liver weight in 500 and 1000mg/kg/b.w./day females and significant decrease in relative brain weight in 1000mg/kg/b.w./day females. A significant increase in relative brain weight in 1000mg/kg/b.w./day was observed in females at the end of recovery period compared to vehicle group. The changes were considered incidental and did not correlate with histopathology findings.

Histopathology: No test item related changes were observed in histopathological assessment across the study groups including male and female rats.

Thyroid estimation: A statistically significant increase of T4 in 500 and 1000mg/kg/b.w./day main group males; and TSH in

1000mg/kg/b.w./day main group males; and T3 in 1000mg main group females and T4 in 250mg/kg/b.w./day main group females was observed as compared to vehicle group. In recovery group, 1000mg/kg/b.w./day dosed group males showed significant increase in T3, T4 and TSH as compared to vehicle group. No correlative adverse observations in gross pathology, organ weights, histopathology for thyroid, brain and liver were observed which are the main organs involved in thyroid endocrinology for the corresponding dose groups and day of analysis. Hence, these changes observed in males are not toxicologically relevant. A decrease in TSH is usually accompanied by an increase in T3, T4 or T3 alone or an increase in TSH is accompanied by reduced T3, T4. This condition was not observed in the present study indicating that the results were atypical.

Mutagenicity study

Based on solubility and by pre-experiment performed with TA 100 strain and *E. coli*, 100 to 1600µg/plate concentration was considered for main experiments in both with or without metabolic activation systems. The first experiment was performed with six concentrations of test item (16, 50, 160, 500, 1600 and 5000µg/plate) along with the negative, vehicle and concurrent positive controls with the five strains i.e. *S. typhimurium* TA 1537, TA1535 and TA98, TA100 and *E. coli* WP2 uvrA pKM101by the plate incorporation assay. No substantial increase in the revertant colony count in any of the five strains were reported at any of the test concentrations in the presence or absence of metabolic activation (S9 mix). Positive controls resulted in significant increases in the revertant count. The spontaneous reversion rates in the negative and positive control were within the range of historical data. In the second experiment, no biologically relevant increase in the revertant counts was observed in any of the five tester strains pre-incubated with the test item. The results of these investigations suggest that under the experimental conditions, CU+ did not induce gene mutation (non-mutagenic) through nucleotide pair changes or frameshifts in the genome of the strains used.

Micronucleus study

The dose of 2000mg/kg/day was selected as the highest dose level for the main study based on the initial dose range finding study showing a decrease in the ratio of polychromatic erythrocytes to total erythrocytes. There were no significant observations found in clinical signs and body weight changes across all groups. There was no significant cytotoxicity observed in the CU+ groups as compared to vehicle group. However, 2000mg/kg/day showed cytotoxicity in bone marrow cells through a reduction in the ratio of polychromatic erythrocyte to total erythrocytes. No biologically or statistically significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in CU+ groups (500, 1000 and 2000mg/kg/day) as compared to vehicle group. The positive control cyclophosphamide monohydrate at 40 mg/kg/day induced a statistically and biologically significant increase in micronucleus frequency. Thus, CU+ was considered non-genotoxic as it did not induce micronuclei in mice bone marrow cells up to 2000 mg/kg dose level under the tested conditions.

Chromosomal aberration study

Based on the observed cytotoxicity on cells during pre-experiments, three non-toxic concentrations of CU+; 0.03125, 0.0625 and 0.125mg/mL were selected for short-term and long-term treatments for the main study. For short-term exposure cultures without metabolic activation system S9, the observed mean % Relative Increase in Cell Counts (RICC) at the CU+ at concentrations of 0.125, 0.0625 and 0.03125mg/ml and positive control was 66.807, 71.227, 78.571 and 75.630% respectively, whereas, the observed % RICC with S9 was 67.548, 73.597, 78.226 and 68.758 %, respectively. For long-term exposure, the observed % RICC in the absence of S9 at the test item concentrations of 0.125, 0.0625 and 0.03125mg/ml of culture and positive control was 61.655, 68.675, 73.703 and 70.490 %, respectively when compared with concurrent vehicle controls. During the main study, 150 well spread metaphases per culture (i.e. 300 metaphases per concentration and controls) were scored for evaluation of frequency of structural chromosomal aberrations.

None of the CU+ treated culture showed any biologically relevant or statistically significant increase in the frequency of aberrated metaphases as compared to vehicle control group with or without - S9. For short-term exposure cultures without S9, the observed mean % aberrated cells at the test item concentrations of 0.125, 0.0625 and 0.03125 mg/ml of culture and positive control were 1.67, 2.34, 2.34, and 9.33 %, respectively, whereas, in cultures with S9, 2, 2.67, 2 and 8.34 % of aberrated cells were observed. For long-term exposure culture the observed mean % aberrated metaphases at the test item concentrations of 0.125, 0.0625 and 0.03125mg/ml of culture and positive control were 1.67, 2, 1.67 and 8.67 % respectively when compared with concurrent vehicle controls. The positive controls exhibited a statistically and biologically significant increase in the frequency of aberrated metaphases when compared with concurrent vehicle controls. The above findings conclude that CU+ did not induce significant structural chromosomal aberrations in CHO-K1 cells under tested conditions.

Discussion

Turmeric extract containing curcumin is one of the most consumed spices in world particularly in India and Asia and has been used in traditional medicine for centuries and safety has been established through multiple human and experimental studies [6,7]. CU+, a highly bioavailable curcumin formulation, was tested for its safety through acute and sub-chronic studies, bacterial revertant assays, *in vivo* mammalian erythrocyte micronucleus test, and *in vitro* mammalian chromosomal aberration test. The acute oral toxicity study results suggest CU+ is non-toxic at dose as high as 2000mg/kg body weight (b.w.) with LD50 cut-off value of 5000mg/kg b.w. Based on the sub-chronic study findings, NOAEL of CU+ was found to be 1000mg/kg b.w./day when administered to Sprague Dawley rats for 90 days through an oral route followed by 28-day recovery period under the conditions tested. The bacterial reverse mutation test showed that the CU+ was non-mutagenic. The *in vivo* mammalian erythrocyte micronucleus test in mice revealed CU+ was non-genotoxic and did not induce significant micronuclei

in mice bone marrow cells at up to 2000mg/kg dose level under the tested conditions. Further, the *in vitro* chromosomal aberrations test showed no significant structural chromosomal aberrations with CU+.

Several *in vitro* and *in vivo* studies have reported safety of turmeric extracts and its standardized powders. Curcumin which is major constituent of turmeric has dose dependent toxic effects [19]. Therefore, many studies explored the safety of turmeric constituents. An acute toxicity study in Swiss albino mice administered with 3000mg/kg b.w. turmeric extract induced some nervous stimulation [20]. In a study in which oral administration of turmeric essential oil to Wistar rats at single doses of 0.1, 0.25, and 0.5g/kg body weight did not have acute toxicity and no mortality was observed, and no effect on alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activity observed as compared to animals that did not receive turmeric essential oil. In addition, there were no abnormalities in urine tests [21]. A single dose study with an oral dosage of 5,000mg/kg b.w. administered to Swiss albino mice and rats did not show any adverse effects during fourteen days [14].

Another acute oral toxicity study of single dose of 5000mg/kg b.w. also did not show any significant change in adverse effects or body weight gain or pathological effects [22]. A study on Swiss mice which was administered 0.2% or 1% turmeric showed toxic effects on liver and liver weight decreased in the mice that received 0.05% ethanolic turmeric extract orally for 14 days [23]. However, there were no abnormal histological studies observed in lungs, brain, and stomach in the turmeric treated animals. The study revealed few histological changes in kidney and spleen. No changes in serum creatinine and urea were observed in those animals. Also, significant increase of serum glutamic oxaloacetic transaminase activity was observed in some animals that were exposed to 0.05%, 1% or 5% turmeric ethanolic extracts. Furthermore, significantly increased serum glutamic pyruvic transaminase activity was observed in 5% turmeric exposed mice. Likewise, our acute or sub chronic studies' results did not show any significant adverse effects.

Several mutagenicity and genotoxicity studies have been reported for turmeric at different concentrations [21,22,24-26] Curcumin can generate reactive oxygen species as a pro-oxidant in the presence of transition metals in cells, resulting in DNA injuries and apoptotic cell death. The pro-oxidant action of curcumin may be related to the conjugated β-diketone structure of this compound [27]. Hence, exploration of mutagenicity and genotoxicity of turmeric extracts or its constituents are necessary. In AMES study tested with the ethanolic extract of turmeric concentrations of 50, 100, and 200ug/plate using *Salmonella*, before and after activation with mammalian microflora and hepatic microsomal enzymes, showed no mutagenicity [25]. Similarly, turmeric essential oil did not induce any mutagenicity in *Salmonella typhimurium*. Furthermore, no DNA damage or chromosomal aberrations was observed when turmeric essential oil was administered orally 1g/kg b.w. for 14 days [21]. In a study, a 5000μg/ml of turmeric polysaccharide extract, dosage did not induce toxicity or mutagenicity in bacteria and no

chromosomal aberration was observed either in human blood lymphocytes between test concentrations of 250.36 to 2500μg/ml of turmeric polysaccharide extract [22].

Additionally, a study using Swiss albino mice orally administered with 0.015% curcumin consisting 0.5% turmeric for 12 weeks and 0.05% turmeric did not show any abnormalities in number and structure of bone marrow chromosomes, and also, mutagenic effect was not observed [26]. We conducted AMES test for mutagenicity and chromosomal aberrations and micronuclei tests genotoxicity and found that CU+ did not induce mutagenic or genotoxic effects. Different formulations are developed for increasing bioavailability of curcumin [7,28,29]. A study investigated nano-formulated curcumin through oral administration for 14 days did not show any fatality and unusual adverse effects in rats [30]. Additionally, Holtzman rats that were exposed to 100mg/kg curcumin nanoparticles orally once a day for 28 days did not show any mortality or body weight change or other haematological or biochemical abnormalities [30]. Another formulation prepared using solid lipid curcumin particle also revealed no abnormality in body weight gain or toxic or pathological effects in animals [31]. Another study examined acute and chronic toxicities of a modified solid dispersion of curcumin-loaded nanocomplexes in gums using mice and hamsters [32].

The report concluded that low or medium doses of their test article was safe in the animal models for both acute and chronic administration. However, they observed that their test articles have the potential to produce toxicity in high-dose treatments, but most abnormal parameters returned to normal levels by 28 days after the final dose. The nanoparticle complexes are used for prolonged and sustained release of curcumin and the components in nanocomplex might produce toxicity in higher doses. For example, the changes in the blood glucose level increases and subsequent cellular damages might be due to overdose of the cellulose based materials present in the nanocomplexes [33,34]. Traditional uses of curcumin reveal a single dose up to 8000 mg was not found in serum and can be administered over long-term without any side effects [35]. Oral administration of CU+ to experimental animals did not show any significant toxic effects from our studies. Our cell culture studies also revealed no significant mutagenic or structural chromosomal aberrations or micronuclei formation.

Conclusion

The current study reports that CU+ is non-toxic when administered orally with LD50 cut-off value of 5000 mg/kg b.w. and NOAEL of 1000mg/kg b.w. per day. The test product did not produce any toxicity as seen from the results of the studies including biochemical assays, body weight changes, and histopathology. Additionally, there were no mutagenicity or genotoxicity observed from the results. Hence, CU+ is safe for oral consumption.

Author Contribution

Conceptualization, AM, MP and PP; methodology, analysis, data curation, RR, JK, DK, SK, SU, SPRT, SRK; writing - original draft preparation, RR, JK, DK, SK, SU, SPRT, SRK.; writing - review and

editing, SRK, AM, MP and PP; project administration, RR, JK; funding acquisition, RR. All authors have read and agreed to the published version of the manuscript.

Declaration of Conflicting Interest

Funding

Institutional Review Board Statement

The studies were approved by Committee for the Control and Supervision of Experiments on Animals (CCSEA) with registration number 1117/PO/RcBiBt-S/RcBiNRC-L/07/CPCSEA.

Data Availability Statement

Data are available from corresponding author upon request.

Acknowledgment

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