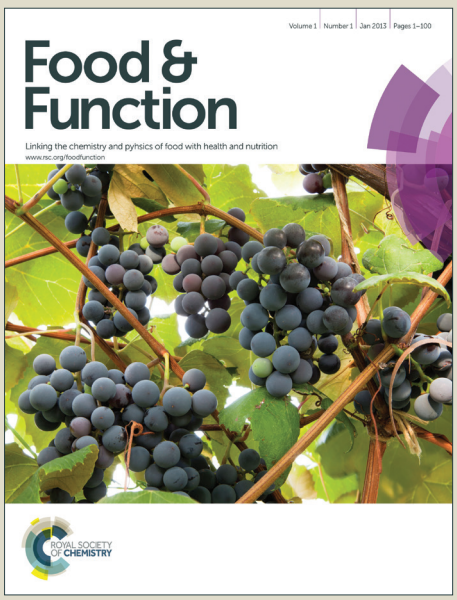
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Enhanced bioavailability and safety of curcumagalactomannosides as dietary ingredient

Vijayasteltar B. Liju[a], Kottarapat Jeena[a], Dinesh Kumar[b], Balu Maliakel[b], Ramadasan Kuttan[a], Krishnakumar IM[b]*

[a] Department of Biochemistry, Amala Cancer Research Centre, Amala Nagar-680555, Thrissur, Kerala, India.

[b] Research and Development Centre, Akay Flavours & Aromatics Pvt Ltd, Cochin - 683561, Cochin, Kerala, India

*** Address for Correspondence:**

Dr. Krishna Kumar I M
R & D Centre, Akay Flavours & Aromatics Ltd
Ambunadu, Malayidamthuruthu P O
Cochin- 683561, INDIA
Tel: +91 484 2686111
Fax: +91 484 2680891
E mail: krishnakumar.im@akay-group.com

Abstract

In spite of the various bioavailable formulations of curcumin for pharma and dietary supplement applications, food grade formulations suitable as a dietary ingredient capable of providing significant levels of plasma curcumin are limited. The present contribution describes the safety and oral bioavailability of a novel water soluble formulation of curcumin, curcumagalactomannosides (CGM), when used as a dietary ingredient in selected food items. CGM was prepared using a food grade hydrocolloid (galactomannans) derived from the kitchen spice fenugreek (*Trigonella foenum graccum*), without using any synthetic excipients. The safety of the formulation was assessed through acute and subchronic toxicity studies on Wistar rats and genotoxicity studies. The efficacy of CGM as a bioavailable dietary ingredient was assessed by successfully preparing various food items and by measuring the post-blood plasma curcumin levels at various time intervals, after the consumption of food items. High performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA) and electrospray ionization tandem mass spectrometer (ESI-MS/MS) was employed for the quantification of plasma curcuminoids. It was observed that CGM is safe and suitable for further development and clinical studies with a no observable adverse effect level (NOAEL) up to 2.0 g/kg/day b.wt. CGM was found to offer seven to ten times higher bioavailability of curcumin in humans, when incorporated into various food/beverage items at 100 mg CGM *per* serving size, as compared to the standard unformulated curcumin.

Key words: Curcumin · Bioavailability · Functional food · Fenugreek fiber · Subchronic toxicity · Mutagenicity.

1. Introduction

Curcuma longa L. (Turmeric) is a popular kitchen spice widely using in Indian curries and medicinal preparations for thousands of years. Modern scientific research have identified many bioactive compounds in turmeric rhizomes in which curcumin or diferuloylmethane, [1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione], a hydrophobic polyphenolic compound, has been characterized as the most active component responsible for numerous health beneficial pharmacological effects.[1,2] Observational studies have already delineated the dietary intake of turmeric with reduced incidence of chronic diseases like Cancer and Alzheimer's in the subcontinent of India.[3,4] With more than 3000 preclinical investigations on various aspects of cancer, curcumin stands first as one of the best studied natural products to date; a great promising candidate capable of selectively modulating multiple cell signaling pathways.[1-4] Considering the multi-targeted mechanism of action of this promiscuous natural agent of immense therapeutic value, curcumin emerged as an 'yellow gold.'[5] Though some food/beverage products containing curcumin are available today, the number of branded food products containing physiologically relevant amounts of curcumin per serving is limited due to the very low solubility and stability of curcuminoids in water and oil.[2,6] The solubility of curcumin in aqueous buffer at pH 5.0 was reported to be as low as 11ng/mL.[6] In addition, the poor systemic oral bioavailability of curcumin, (due to its extremely low aqueous solubility, high hydrolytic instability and rapid enzymatic *in vivo* degradation), was also a major limitation against the development of curcumin as a functional food ingredient or therapeutic agent.[2,6,7]

While the uptake and distribution of curcumin in body is essential for its biological activity, curcumin offered only negligible quantities in plasma due to the poor absorption, permeability and rapid metabolism.[2,7] Consumption of even 10 to 12 g of curcumin as capsules was reported to provide only less than 50 ng/mL of curcumin in human plasma.[8] Many attempts to overcome the problem of poor bioavailability of curcuminoids, comprising the use of adjuvants to inhibit *in vivo* enzymatic degradation, formation of nanoparticles, and formulation of phospholipid complexes and liposomes were reported.[2,7,9] However, enhanced bioavailable formulations suitable for development as food ingredient are limited due to the presence of non-food grade excipients, extreme insolubility/hydrophobicity, nano-size, regulatory noncompliance and high cost. Thus, there exists a huge demand for novel technologies and formulations to produce cost-effective food-grade bioavailable formulations for stable and water soluble curcuminoids employing GRAS (Generally Regarded as Safe)-listed natural ingredients.

Recently, we reported an enhanced bioavailable form of curcumin, namely curcumagalactomannosides, (*hereinafter named as* '*CGM*'), formulated using fenugreek (*Trigonella foenum-graecum*)-derived soluble dietary fiber composed of galactose and mannose units (galactomannan).[10] Fenugreek galactomannans have already established as a versatile hydrocolloid possessing gelling, thickening, emulsifying, stabilizing and encapsulating properties[11], with functional benefits such as hypolipidemic, hypoglycaemic, appetite reducing and gastroprotective effects.[12] CGM was found to deliver significantly high levels of plasma curcumin levels (1 to 1.5 μM for

6 to 8 h post-administration) as compared to standard unformulated curcumin (0.04 to 0.08 μM for less than an hour) when supplemented at 250 mg/kg b.wt. in Wistar rats and 250 mg in humans.[10] Thus, the formulation CGM, is a highly economical, hydrocolloid-based, regulatory complaint, enhanced bioavailable form of curcumin having the potential for further development as a novel functional food ingredient, if it can further satisfy the other requirements on taste/flavor, solubility, stability, microbial status, safety etc. In the present contribution, solubility, particle size, stability (storage, pH and heat), nutritional composition and safety as studied by acute, subchronic and genotoxicity studies were investigated to check its possibility of development as a food ingredient capable of delivering significant amounts of curcumin into blood stream. Optimized dosages of CGM for incorporation in selected food/beverage items, without compromising the taste/appearance and bioavailability, was also investigated in the present study. HPLC-PDA and tandem mass spectrometry (ESI-MS/MS) were employed for the quantification of free curcuminoids in plasma and the area under curve (AUC) calculations of total free curcuminoids concentration-time plots was considered for bioavailability estimations. Unformulated curcumin standardized to 95% purity was supplemented as (500 mg × 2) hard shell gelatin capsules for the purpose of comparing the bioavailability, since it was not possible to incorporate the unformulated curcumin into the food and beverage recipes investigated in the present study.

2. Materials and methods

2.1 General

Curcumagalactomannosides (CGM), exhibiting enhanced bioavailability (prepared as per the method of Krishnakumar et al., 2012; *see reference,* 10) and unformulated curcumin standardized to 95% purity were provided by Akay Flavours & Aromatics Pvt Ltd, Kerala, India. Standard curcumin (CAS Registry No. 458-37-7) was purchased from Sigma-Aldrich, Bangalore, India. Curcumin content was estimated by a reverse phase high pressure liquid chromatography (HPLC) procedure, employing a phenomenex column (250 x 4.6 mm, 3 µm) (Shimadzu Analytical Pvt. Ltd, Mumbai, India).[12] Agilent 6460 triple quadrupole tandem mass spectrometer (MS/MS) fitted with an electrospray ionization (ESI) probe (Agilent India Pvt. Ltd, Bangalore, India) was employed for the identification of curcuminoids in plasma using multiple reaction monitoring mode (MRM). Agilent JetStream source operated with a capillary voltage of 3500 V, 375^{o}C sheath gas temperature and 325^{o}C drying gas temperature was employed in positive ionization mode. MRM for each of the curcuminoids were recorded at collision energies 48, 24 and 20 V respectively for curcumin, DMC and BDMC with the corresponding fragmentor energies of 113, 103 and 94 V. Analytical reference standards of curcumin (CAS# 458-37-7; purity >98%) DMC (CAS# 22608-11-3; purity >98%) and BDMC (CAS# 33171-05-0; purity >95%) were obtained from Sigma-Aldrich, Bangalore, India. Nutritional composition was analysed by standardised procedure of Association of Analytical Chemists.[14] Particle size was measured by a Melvern Zetasizer Nano ZS90 particle size analyzer (Malvern Instruments Ltd, Worcestershire, UK).

2.2. Stability studies of CGM

Storage stability studies were carried out using a protocol prepared by following the International Conference on Harmonization (ICH) guidelines.[15] Briefly, the sample packets (10 g) of CGM were incubated at 40°C ± 2°C and 75 ± 5% relative humidity for a period of 6 months in a stability chamber (Remi, Mumbai, India). The samples were withdrawn at 0, 1, 2, 3, and 6 months and analyzed for various physicochemical parameters as shown in Table 1. The pH stability of aqueous soultion was checked by preparing 5% (w/w) solution at pH 2.0, 5.0, and 6.8 using hydrochloric acid and phosphate buffers. 5 mL solutions were withdrwan at regular intervels of 2 h for a period of 24 h and the curcumin content was checked by HPLC. Temperature stability was checked by keeping the solution at 90 ± 2°C for 30 min followed by HPLC analysis.

2.3. Animals

All animal experiments were conducted after getting prior permission from Institutional Animal Ethics Committee and were performed as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Young adult male (average b.wt. 200 g) and female (average b.wt. 150 g) Wistar rats, 7-8 weeks old, were purchased from Animal Breeding Station, Kerala Agricultural University, India. They were housed in the animal house facility of Amala Cancer Research Centre, in well ventilated sterile polypropylene cages. Rats were maintained at a controlled temperature, 22 ± 2°C and relative humidity 60 ± 5% and provided 12 h light/dark

cycles. They were fed with normal pelleted rat chow (M/s Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*.

2.4. Toxicity studies of CGM

2.4.1. Acute toxicity studies

Acute toxicity studies were performed initially to check whether CGM produced any toxic effect when supplemented at maximum recommended concentrations. CGM was suspended in water and orally administered as single dose at the limit dose of 5 g/kg b.wt in a sequential manner. Forty rats were divided into four groups such that each group contains five animals per sex with similar weight. Group I was the control (untreated) and Groups II, III and IV were administered with CGM at 1.0, 2.0 and 5.0 g/kg b.wt respectively. All the animals were observed for mortality, clinical and behavioral signs for first 10, 30, 60, 120, 240 and 360 min post dose and thereafter twice daily for mortality and once daily for clinical signs, during the study period of 14 days.

2.4.2. Oral subchronic toxicity study (90 days).

Fifty healthy Wistar rats after acclimatization to laboratory conditions were divided into five groups as above. Group I was kept untreated normal, group II as vehicle (1 mL water) treated control and groups III, IV and V treated with CGM at doses of 0.5, 1.0 and 2.0 g/kg b.wt respectively, by oral gavage for 90 days. Observations of pharmacotoxic signs were made at 10, 30, 60 and 120 min post-dose during the first day and continued twice daily for 90 days. The animals were monitored for clinical and

behavioral symptoms such as diarrhea, immobility, neuromuscular problems, mortality and any other adverse reactions. Body weight, food and water consumption were determined every week and expressed for a single cage of five animals. After 90 days, the animals were sacrificed by cervical dislocation under light ether anesthesia. Necropsy of the animals was performed in the presence of a veterinary doctor and observations were recorded. The weight of the brain, liver, stomach, kidney and spleen were recorded and expressed in relation with the final body weight. The tissue samples were fixed in 10% formalin and embedded tissues were cut into slices of 2 to 4 µm and stained with hematoxylin-eosin for histopathological examinations with an optical microscope of 100X magnification (Olympus-Magnus trinocular microscope, Tokyo, Japan).

Blood was collected by direct heart puncture and taken in the EDTA vials for assaying haematological parameters. Total white blood cells were measured after diluting the blood in Turk's fluid and counting them using a hemocytometer.[15] For differential leukocyte count, blood smear was prepared on a clean glass slide, stained with Leishman's stain and various types of cells were counted manually using a microscope.[16] Platelet count was determined by diluting the blood with Rees Ecker diluting fluid and counted using a Haemocytometer.[16] Total RBC count was measured by diluting the blood with Dacie's fluid and counted using a counting chamber.[16] Haemoglobin content (Hb) was measured by cyanmethaemoglobin (Drabkin's) method using the kit purchased from M/s Agappe Diagnostics, Mumbai, India.

A part of the blood was also collected in non-EDTA tubes and serum was separated after centrifugation at 5000 rpm for 10 min and was used for the following investigations. Total bilirubin was determined as detailed by Pearlman method;[17] alkaline phosphatase (ALP) was estimated by *p*-nitrophenyl picolinate (PNNP) hydrolysis; alanine amino transferase (ALT) and aspartate aminotransferase (AST) were estimated by kinetic method kits supplied by M/s Raichem, India using a Microlab 300 auto analyzer (Merck, Mumbai, India); albumin was determined by its reaction with bromocresol green and total protein concentration was determined by Biuret method.[18] Kidney function markers such as creatinine and blood urea nitrogen were estimated by Jaffe-Kinetic and Urease method respectively.[19] Total cholesterol was estimated by CHOD–PAP (cholesterol oxidase–phenol+aminophenazone) enzymatic method,[20] triglycerides by GPO–PAP (glycerol-3-phosphate oxidase–phenol+aminophenazone) method[21] and HDL cholesterol by precipitation with phosphotungistic acid. VLDL cholesterol was estimated by the Friedewald equation (VLDL= triglyceride/5) and LDL cholesterol by calculation as LDL = total cholesterol - (HDL+VLDL). Serum sodium, potassium and bicarbonate were estimated using a Flame photometer with an ion selective electrolyte analyzer. Chloride was estimated by mercurousthiocyanate method using a kit from M/s Raichem, India.

2.4.3. Mutagenicity studies

Evaluation of the ability to induce reverse mutation at the histidine loci in three *S. typhimurium* strains of TA 98, TA 100 and TA 102 (Ames test) was conducted according to standard procedures.[22] Mutagenicity of CGM was done by plate

incorporation method in the presence and absence of an exogenous metabolic activation system at five doses (0.1, 0.5, 1, 3, 5 mg/plate) in triplicates for each dose. 5 µg sodium azide/plate dissolved in distilled sterile water was used as positive control. A plate without drug and mutagens was used as negative control and 200 µl DMSO was used as vehicle control. 2 mL top agar layer (0.6% agar and 0.5% NaCl) containing *S. typhimurium* strains, 0.5 mM histidine/biotin solution and different concentrations of CGM were shaken well and poured on to 25 mL of agar. The plates (triplicate) were incubated for 48 h at 37^0C and revertant colonies were counted using colony counter.

Rat liver microsomal enzyme was used for metabolic activation of mutagen *in vitro.*[23] Microsome P450 enzyme was induced in rat liver by administration of 0.1 % phenobarbital and liver was excised aseptically and microsomal S9 fraction was prepared by mixing S9 mix (500 µL) with sodium phosphate buffer (0.2 M, pH 7.4), NADP (0.1 M), glucose 6- phosphate (1 M, pH 7.4), $MgCl_2$–KCl (10 µL) in presence of mutagen, 2-acetamidoflourene (20 µg/plate), different concentrations of CGM and bacterial strains TA 98, TA 100, TA 102 and incubated at 37^0C for 45 min. Further it was mixed with 2 mL of molten top agar, supplemented with histidine and biotin (0.05 mM). The mixture was shaken well and poured on to the surface of 25 mL of minimal agar. After 48 h incubation, the mutagenic response was evaluated by counting the revertant colonies per plate and comparing with the control groups. The test substance was considered to be mutagenic if there is a three-fold increase in the tester strains when compared to the negative control.

2.5. Preparation of food containing CGM and its oral bioavailability studies

100 mg/serving size of CGM containing food items were successfully prepared and their organoleptic properties were assessed by a panel of seven experts. On the basis of sensory results, following food items were used for consumption and subsequent estimation of bioavailability of curcumin from the food stuff. Vegetable soup premix powder containing 100 mg of CGM *per* 10 g was prepared and made into 100 mL soup with 60 to 65^{o}C hot water; 20 g of mango juice pulp was homogenized with 100 mg of CGM and further diluted to 100 mL with cold water; 100 mg of CGM containing 30 g yogurt, honey containing 100 mg of CGM *per* 10 g, chocolate containing 100 mg of CGM *per* 20 g and 20 g of cookies containing 100 mg of CGM were selected for studies.

Eight human volunteers (four male and four female) aged between 25 and 40 years, who were healthy and not involved in any medication or health supplementation was selected for bioavailability studies. Institutional ethical committee clearance (IEC) and the written consent from all individuals were obtained before the study, as per the protocol suggested by the Government of India. The volunteers were not allowed to take turmeric containing food for three days prior to the test. Each volunteer was first given 1000 mg (500 mg x 2 hard gelatin capsules) of unformulated standard curcumin as the placebo, since it could not be incorporated in the above mentioned food items. 2 mL blood samples were withdrawn at 0, 0.5, 1, 3 and 5 h post-dose; plasma was separated by centrifugation and frozen at -20^{o}C till analysis. After 1 week of curcumin study, the subjects were given one serving of food item containing 100 mg CGM and the protocol was repeated exactly the same as above for collection of plasma samples at

various time in after food consumption. Volunteers were asked to complete the food consumption within 3 min. The same was repeated for each food item at an interval of one week. Total curcuminoids in plasma was measured by a previously reported reverse phase HPLC-PDA-ESI-MS/MS procedure employing PDA detection at 420 nm and methanol as mobile phase; (Phenomenex column 250 × 4.6 mm, 3 μm).[10] The mass spectrometer was operated in positive ionization mode in multiple reaction monitoring (MRM) mode, which could detect *m/z* 369 (369 → 116.9) for curcumin, *m/z* 339 (339 → 146.9) for DMC and *m/z* 309 (309 → 146.9) for BDMC.

2.6. Statistical analysis

The values are expressed as mean ± SD. The statistical significance was compared between untreated and experimental groups by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnet multiple comparison test) using Graphpad in Stat software (version 3.05). Data on CGM treated animals were compared with untreated animals. The pharmacokinetic parameters were calculated by linear regression using Microsoft Excel (Microsoft Corporation, Richmond, VA, USA)

3. Results and discussion

Dietary intake of curcumin, the yellow pigment in turmeric rhizomes, has witnessed a tremendous growth during the last decade owing to the increasing number of research based evidences demonstrating its safety and pharmacological benefits as an efficient natural agent having multi-targeted mechanism of action against various types of disease states including cancer and Alzheimer's.[1-4] However, the poor oral

bioavailability of curcuminoids remains as a limiting factor during the attempts to translate most of the preclinical results to the clinic.[2] The new formulation of curcumagalactomannosides, (CGM), has already shown to exhibit more than 20-fold enhancement in bioavailability as compared to the standard curcumin, when supplemented as capsules.[10] Being a combination of bioactive extracts of two popular kitchen spices (curcumin and fenugreek) without any organic solvents or additives, the present contribution reports the other properties of CGM such as solubility, stability, particle size and safety to exploit the possibility of use as a food ingredient capable of providing significant levels of plasma curcuminoids. Various food/beverage items impregnating CGM at various dosages per serving size were also prepared and analyzed for sensory perception. The efficacy of such food items to provide high plasma curcumin levels (bioavailability) upon the consumption of a serving size of such a food was also investigated as proof of the concept.

Enhanced bioavailable formulation of curcumin (CGM) was prepared from 95% pure commercially available curcumin consisting of 78% curcumin, 16% demethoxycurcumin, 6% bis-demethoxycurcumin (commonly referred to as '*curcuminoids*') and fenugreek derived soluble dietary fiber containing 76% galactomannans and 16% proteins. An earlier reported procedure of gel-phase dispersion technique was employed for the preparation of CGM.[10] CGM was obtained as a free flowing powder with 100 to 200 μm particles capable of swelling and dissolving in water to produce a homogeneous and viscous yellow solution consisting of colloidal particles of 1.2 ± 0.2 μm. Swelling capacity of CGM was measured as 20.2 mL/g in

water at pH 7.0, indicating the high water holding capacity. It was found to have a nutritional composition of 46.9 % dietary fiber, 6.0% protein, 2.8% fat, 2.3% moisture and 40.2% curcumin with an energy content of 64.4 Cal/100 g.

3.1. Stability of CGM

When tested for storage stability under accelerated conditions as specified on ICH guidelines, CGM was found to cause only 2.1% loss in curcumin content with no significant change in other parameters like colour, appearance, odour, taste, moisture content, bulk density, carbohydrates, proteins, and microbial status (Table 1), indicating the possibility of convenient storage for two years at ambient conditions of less than 25^{o}C in closed containers, away from direct light and moisture. An aqueous solution of CGM at pH 7.0 showed only 3% curcumin degradation when stored for 21 days under room conditions of 28±2^{o}C. On heating to 90±2^{o}C for 30 min, the solution showed only 0.1% of curcumin degradation. Thus, the observed solubility, stability and enhanced bioavailability of CGM along with the possibility of its economic production on a commercial scale and regulatory compliance is the basis of the present study to further evaluate its potential as a novel functional food ingredient capable of delivering significant levels of curcuminoids into blood stream.

3.2. Toxicity studies of CGM

A systematic investigation of the safety of CGM as studied by acute, subchronic and genotoxicity was carried out, since these studies are generally used to satisfy hazard classification and labelling requirements for risk assessment in human health and

environment.[24] The application of ultrasound as a novel method of preparation of curcumagalactomannosides (CGM) particles, and its subsequent oral delivery to produce significantly high levels of colloidal curcumin in the intestine for better absorption leading to longer duration of significantly high levels of curcumin in the blood demanded the pharmacological safety evaluation of CGM for human use. Moreover, the systematic evaluation of toxicity of fenugreek galactomannans as employed in the preparation of CGM has not been reported, though the toxicity studies of debitterized fenugreek powder containing more than 60% fiber content has been reported.[25] However, curcumin has been evaluated in detail and reported as safe even at high doses.[8] Though an initial investigation on 28-days repeated dose toxicity studies of CGM on Wistar rats has shown no adverse effects,[10] the present study evaluated the oral acute (14 days), subchronic (90 days) and genotoxic effects of CGM.

3.2.1 Acute toxicity studies

The administration of a single high dose of CGM up to a concentration of 5 g/kg b.wt did not produce any mortality, behavioral changes or clinical signs of toxicity either immediately or during the post-treatment period of 14 days, irrespective of the sex of the animals. Food and water intake and body weight change were similar to the normal group of animals indicating no detrimental effect of CGM upon the growth pattern, since these indicators represents the adverse effect of the drugs and chemicals.[26] Necropsy of the animals showed neither any morphological changes in the tissues nor any gross pathological abnormalities with no significant change in the weight of organs as compared to the untreated group of animals of both sexes after 14 days of administration. Hepatic function parameters (ALT, AST, ALP, total bilirubin,

total albumin & globulin and A/G ratio), renal function parameters (blood urea, serum creatinine, serum electrolytes), and haematological parameters (WBC, RBC, platelet, haemoglobin, HDL, LDL, VLDL, triglycerides) were also comparable to the normal animals indicating the safety of CGM administration at 5 g/kg b.wt.

3.2.2. Subchronic toxicity studies

Oral administration of CGM for 90 days at doses of 0.5, 1.0 and 2.0 g/kg b.wt did not produce any obvious signs of toxicity or mortality. The body weight and relative food and water intake of the animals treated with CGM were similar to those of untreated group of animals of both sexes (Fig. 1). The average food intake of male rats was nearly 15 g/day/animal and that of female was 10 g/day/animal. Data on the various haematological parameters measured in rats fed with CGM and normal group of animals were given in Table 2, which showed no significant alterations in Hb, RBC, WBC, and differential counts. Haematopoietic system is often considered as one of the most sensitive parameters to assess the toxicity of drugs in animals and humans.[26] Similarly, the data on the biochemical parameters of animals treated with CGM and that of untreated normal group (Table 2) also showed no significant alterations in any of the specific enzyme activities (SGPT, SGOT, ALP) or in bilirubin, total protein and A/G ratio. Since the liver damage is usually accompanied by an elevation of serum SGPT and SGOT, the consumption of CGM was found to have no toxic effect to liver.[27, 28] A similar trend was observed in renal function markers (blood urea, serum creatinine, serum electrolytes) even though higher levels of curcuminoids was providing to the blood stream continuously for 90 days by administering CGM at 2.0 g/kg body weight (Table 2). The data points towards the safety of nephron cell function, since any

damage to nephron cell will be accompanied by an elevation of serum creatinine and urea.[26,28] Similarly, increase in creatinine and urea levels may be attributed either due to the damage to nephron cell function[29] or due to the depletion of extracellular fluid volume which usually prevail during hyperglycemia.[30] Cholesterol, LDL, HDL, triglycerides and VLDL cholesterol levels were also showed no significant changes after thirteen weeks of oral administration of CGM, when compared to the untreated rats of both sex (Table 2). Since cholesterol degradation, glucose synthesis and generation of free glucose from hepatic glycogen are taking place in the liver, this result also indicated the normal functioning of the liver without any adverse effect on the lipid or the carbohydrate metabolism.

Necropsy of the CGM-treated group of animals, immediately after dissection, showed normal appearance of various organs and tissues. There were no significant differences in the group mean absolute or relative weights of various vital organs in CGM-treated rats when compared with untreated animals (Table 1). Histopathology of the brain, kidney, spleen and liver did not show any abnormalities among CGM treated animals indicating the absence of any adverse toxicological effects upon CGM administration at a dose of 2.0 g/kg b.wt for 13 weeks (Fig. 2).

3.2.3. Mutagenicity of CGM

Mutagenic substances can alter the genetic materials and may act as carcinogens. Some act as direct mutagens and some are indirect mutagens needing S9 microsomal fractions for activation. Ames test is a quick and convenient assay to assess the mutagenic potential of a compound and 90% of known carcinogens may be identified

via this test.[21] Though curcumin is known to be non-mutagenic and has reported to inhibit the mutagenicity of several environmental mutagens and carcinogens,[30] the unique composition of soluble dietary fiber and proteins of fenugreek used in the preparation of CGM has not been tested so far for mutagenic effects. It was found that curcumagalactomannosides, CGM), did not produce any revertants in the absence and presence of metabolic activation (S9) mix when tested up to a concentration of 5 mg/plate, as compared to the negative control in *S. typhimurium* strains. The positive control containing known mutagens induced increase in mean number of revertant colonies in each strain, while the vehicle control did not show any increase in revertant colonies (Table 3), indicating that there was no dose related mutagenicity for CGM, either with or without metabolic activation.

3.3. Preparation of food/beverage containing CGM

Selected food items were prepared by incorporating the new formulation of curcumin, CGM, at 100, 200 and 300 mg per serving levels and subjected to sensory analysis by a panel of experts to select the optimum dosage which can be conveniently incorporated into the food matrix without compromising taste and appeal. Though these dosages of CGM could incorporate without issues, the bioavailability study was carried out at 100 mg/serving dosage to check the efficacy of lowest possible dose. 100 mg of CGM incorporated soup premix with an attractive pale yellow colour gave a tasty soup when 10 g of the premix was diluted with 100 mL of water at 60 to 65^{o}C. Fortification in fruit/vegetable juices, juice powders and fruit pulp also gave acceptable organoleptic properties with an enhancement of colour to juices like mango, pineapple, lemon and

orange. The concentration of CGM may be adjusted to get the desired colour hue. A commercially available mango pulp (20 g) was fortified with 100 mg of CGM by homogenization and further diluted to get 100 mL mango juice. Homogenization was found to be suitable to impregnate CGM in honey to produce a desired concentration of 100 mg per 10 g of honey. Yet another matrix investigated for impregnation of CGM was chocolate. CGM was added along with cocoa solids and further grinded to produce chocolates containing 100 mg CGM per 20 g, with an attractive dark brown characteristic chocolate colour. It was also added to milk chocolates to produce natural yellow coloured chocolate of lemon shade. When tried on yogurt and milk, a slight settling of curcuminoids was observed. However, the problem was solved by high pressure homogenization in presence of a thickening agent, pectin. Finally cookies were also prepared at 100 mg CGM per 20 g, to get brownish yellow cookies. Though there were no taste or aroma issues, the baking process at 175^{o}C for 20 min for cookies was found to degrade the curcuminoid levels by 33%.

3.4. Bioavailability studies

The relative absorption of curcuminoids upon consumption of CGM incorporated food was tested on human volunteers, by quantifying the curcuminoids levels in blood plasma at various intervals of time after the ingestion of one serving size of the food item containing 100 mg of CGM. The identity of curcuminoids in plasma was confirmed by spiking standard curcumin in animal plasma at 1.0 μg/mL followed by extraction of curcuminoids and positive ion electrospray tandem mass spectrometric analysis in MRM mode. Consumption of food/beverage containing CGM was found to

significantly ($p < 0.05$) enhance the plasma curcuminoids levels, as compared to the standard curcumin with 95% purity, when administered as 1000 mg (500 mg × 2) hard shell gelatin capsules (Fig. 3). The area under curve (AUC) calculations of the plasma curcumin concentration – time plot revealed a relative enhancement of 7.2 times for soup, 5.8 times for yogurt, 6.8 times for mango juice, 8.7 times for honey and 7.4 times for chocolate when consumed as a single serving containing 100 mg of CGM. AUC is regarded as the robust method of measurement of bioavailability, since it take into consideration of the entire response over time, rather than the maximum concentration of curcumin observed in the blood at a particular time point, 'C_{max}'. [32+] However, it was important to note that no curcumin could be detected upon consumption of 20 g of cookies containing 100 mg of CGM, which involved a high temperature baking process. The time taken to reach the maximum curcumin concentration in plasma was 30 min after the food ingestion and no curcuminoids were detected after 4 h. Unformulated curcumin, on the other hand, was never absorbed when used in the food items investigated in the present paper, though the uniform impregnation was not achieved due to the insolubility and crystallinity. The solubility of standard curcumin in aqueous solutions is very low and the hydrolytic instability is very high.[6] It is therefore poorly absorbed even with high dosages as high as 12 g/day. [8] The pharmacokinetic parameters including the maximum concentration of curcuminoids found in plasma (C_{max}), the time at which maximum curcumin concentration was found in plasma (T_{max}) and the time taken for 50% of absorbed curcumin to degrade ($T_{1/2max}$) were also estimated (Table 4). It was observed that the C_{max} and $AUC_{0\text{-}6h}$ were significantly higher after the consumption of CGM containing

food, but the C_{max} and $AUC_{0\text{-}6h}$ were different for different food items (Fig. 3). However, all the parameters were significantly high for CGM-impregnated foods as compared to standard curcumin indicating the better bioavailability of curcumin upon the consumption food containing CGM. T_{max} for CGM containing food was 30 to 60 min as compared to 30 min for standard curcumin. Moreover, the absorbed curcumin was found to stay in the blood for longer duration, as evident from the $T_{1/2max}$ vales ranging from 2 to 2.7 h in various food matrices, as compared to standard curcumin (Table 4). Though various bioavailable formulations of curcumin have reported, this is the first report to the best of our knowledge, that the use of a bioavailable formulation of curcumin as a dietary ingredient capable of furnishing curcumin into plasma upon the respective food consumption.

It was observed from the above results that the functionalities of fenugreek soluble fiber/protein composite such as high swelling index in water, gel-like character and gummy nature has helped to establish a strong binding of colloidal curcumin in the fiber network for providing a microencapsulation effect even when present in various food/beverage matrices, which provided protection against the rapid metabolism during the gastrointestinal transit. Moreover, the thickening property of fiber and effect of ultrasonication helped to change the character of the leached curcuminoids into amorphous, water miscible and colloidal form, for better absorption.

4. Conclusions

The present study demonstrated the safety, stability, solubility and enhanced bioavailability of a novel food grade formulation of curcumin, CGM, when used as dietary ingredient. The formulation was prepared by the ultrasound mediated gel-phase impregnation of curcumin into a non-digestible, but fermentable prebiotic soluble fiber - protein composite isolated from the popular kitchen spice fenugreek, without any excipients/additives. The present toxicity study carried out in our laboratory revealed the safety of the acute dosage at 5 g/kg b.wt and subchronic oral administration at 2.0 g/kg b.wt of CGM in Wistar rats with a NOAEL of 2.0 g/kg/day. Ames test demonstrated no mutagenic character to CGM when tested up to a concentration of 5 mg/plate. Further investigations on solubility, particle size, stability and organoleptic properties indicated the possibility of usage of CGM as a functional hydrocolloid-based food ingredient capable of providing enhanced bioavailability for curcumin when incorporated into various food and beverage items at 100 to 300 mg *per* serving size, without compromising the taste/appeal. Considering the encouraging results, systematic toxicity evaluation as per OECD guidelines and optimization of conditions of impregnation of CGM in various food matrices are warranted for further development of this novel ingredient.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are grateful to M/s Spices Board of India for financial support, research grant MKT/R&D/04/10-12, for value addition of spices. Also sincere thanks to Food Application Laboratory of M/s Akay Flavours & Aromatics Pvt. Ltd, for the preparation of CGM incorporated food/beverages.

References

1. I. Shureiqi and J. A. Baron, *Cancer Prev. Res.,* 2011, **4**, 296-298
2. P. Sahdeo, K. T. Amit and B. A. Bharat, *Cancer Res. Treat.*, 2014, **46**, 2-18
3. C.M. Kaefer and J. A. Minler, *J. Nutr. Biochem.*, 2008, **19**, 347–361
4. K. Ramaswamy, C. G. Subash, J. H. Kim, R. Simone and B. B. Aggarwal, *Mol. Neurobiol.,* 2011, **44**, 142–159
5. C. T. Keith, A. A. Borisy and B. R. Stockwell, *Nat. Rev. Drug Discovery,* 2005, **4**, 71-78
6. H. H. Tonnesen, M. Masson and T. Loftsson, *Int. J. Pharm.,* 2002, **244**, 127-135
7. M. M. Yallapu, M. Jaggi, and S. C. Chauhan, *Drug Discovery Today*, 2012, **17**, 71-80
8. C. D. Lao, M. T. Ruffin, D. Normolle, D. D. Heath, S. I. Murray, J. M. Bailey, M. E. Boggs, J. Crowell, C. L. Rock and D. E. Brenner, *BMC Complement. Altern. Med.,* 2006, **7**, 6-10
9. H. Sasaki, Y. Sunagawa, K. Takahashi, A. Imaizumi, H. Fukuda, T. Hashimoto, H. Wada, Y. Katanasaka, H. Kakeya, M. Fujita, K. Hasegawa, and T. Morimoto, *Biol Pharm Bull*, 2011, **34**, 660-5

10. I. M. Krishnakumar, A. Ravi, D. Kumar, R. Kuttan and B. Maliakel, *J Functional Foods,* 2012, **4**, 348-357

11. M. Srivastava and V. P. Kapoor, *Chem. & Biodiv.*, 2005, 2, 295–317

12. J. R. Mathern, S. K. Raatz, W. Thomas and J. L. Slavin, *Phytotherapy Res.*, 2009, 23, 1543-1548

13. B. K. Jadhav, K. R. Mahadik and A. R. Paradkar, *Chromatographia,* 2007. DOI: 10.1365/s10337-006-0164-8

14. W. Horwits and G. W. Latimer Jr. *Official Methods of Analysis of AOAC International*, 18th Edition, Maryland, USA, 2002.

15. Guidance for Industry Q1A (R2). *Stability Testing of New Drug Substances and Products,* 2003. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073369.pdf.

16. D. A. Nelson and M. W. Morrie, *Basic methodology in clinical diagnosis and management by laboratory methods.* (ed) J. B. Henry, Philadelphia, 1984, p. 589

17. G. K. Pal and P. Parvati, *Textbook of practical physiology*, Orient Longman Private Limited, India, 2003.

18. F. C. Pearlman and R. T Lee, *Clin. Chem.*, 1974, **20**, 447–453

19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 1951, **193**, 265-275

20. R. Deeg and J. Ziegenhorn, *Clin. Chem.*, 1983, **29**, 1798–1802

21. T. G. Cole, S. G. Klotzsch and J. McNamara, *Handbook of lipoprotein testing*, (ed) N. Riafi, G. R. Warnick, M. H. Dominiczak, AACC Press, Washington, 1997, pp. 115–126

22. B. N. Ames, J. Mccann and E. Yamasaki, *Mutation Res.*, 1975, **31**, 347–364

23. G. K. Jayaprakasha, B. S. Jena, P. S. Negi and K. K. Sakariah, *Z Naturforsch.,* 2002, C57, 828-835

24. OECD Guidance on acute oral toxicity testing. *OECD Environment Health and Safety publications series on testing and assessment.* No.24. Organization for Economic Co-operation and Development, Paris, 2001

25. Muralidhara, K. Narasimhamurthy, S. Viswanatha S and B. S. Ramesh, *Food Chem. Toxicol.,* 1999, **37**, 831-838

26. S. P. Tofovic and E. K. Jackson, *J. Cardiovasc. Pharmacol.*, 1999, **33**, 360–366.

27. M. F. Rahman, M. K. Siddiqui and K. Jamil, *Hum. Exp. Toxicol.*, 2001, **20**, 243–249

28. J. E. Hilaly, Z. H. Israili and B. Lyouss, *J. Ethnopharmacol.*, 2004, **91,** 43-50

29. N. Lameire, W. Van Biesen and R. Vanholder, *Lancet,* 2005, **365**, 417–430

30. C. E. Mogenson and C. K. Christensen, *Hypertension,* 1985, **7**, 1164 -1168

31. M. Nagabhushan and S. V. Bide, *Nutrition and Cancer*, 1986, **8**, 201-210

32. W. Stahl, H. van den Berg, J. Aruthur and A. Bastl, *Mol. Aspects Med.*, 2002, **23**, 39-100

Legend for Figures and Tables

Fig. 1 Effect of subchronic administration of CGM on (A) body weight (B) food consumption (c) weight of organs of both male and female rats

Fig. 2 Histopathological analysis of rat organs treated with 2 g/kg b.wt. of CGM for 90 days

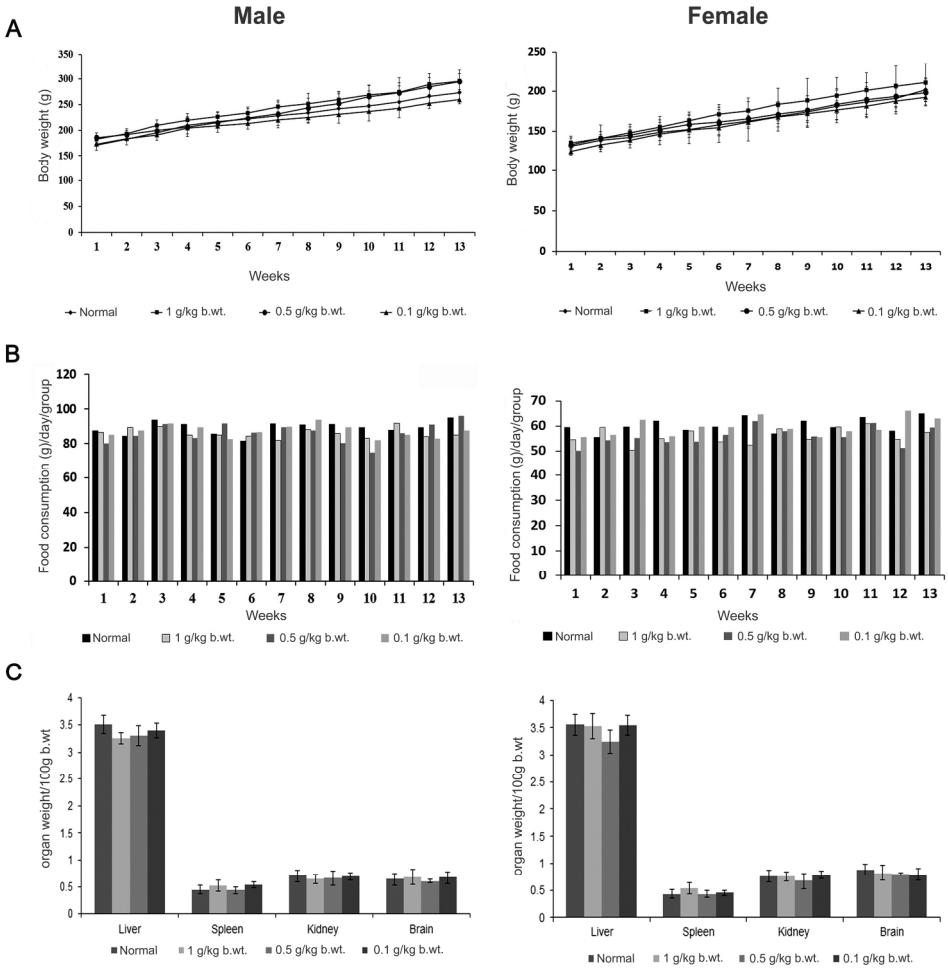
Fig. 3 Average concentration of curcumin observed in human plasma after consumption of various food items containing 100 mg of formulated curcumin, CGM, *per* serving. Serving sizes were 10g soup premix powder in 100 mL hot water, 20 g mango pulp in 100 mL water, 10 g honey, 20g chocolates and 30 g of yogurt. Standard curcumin was administered at 1000 mg dose as hard shell gelatin capsules (500 mg × 2). The data is expressed as the mean ± SD (n = 8); *$p < 0.05$; (100 mg CGM containing food versus 1000 mg standard curcumin)

Table 1 Stability study of *CGM* performed as per an in-house protocol based on ICH guidelines. The samples were incubated at 40°C ± 2°C and 75% ± 5% RH (Accelerated stability study) for a period of 6 months

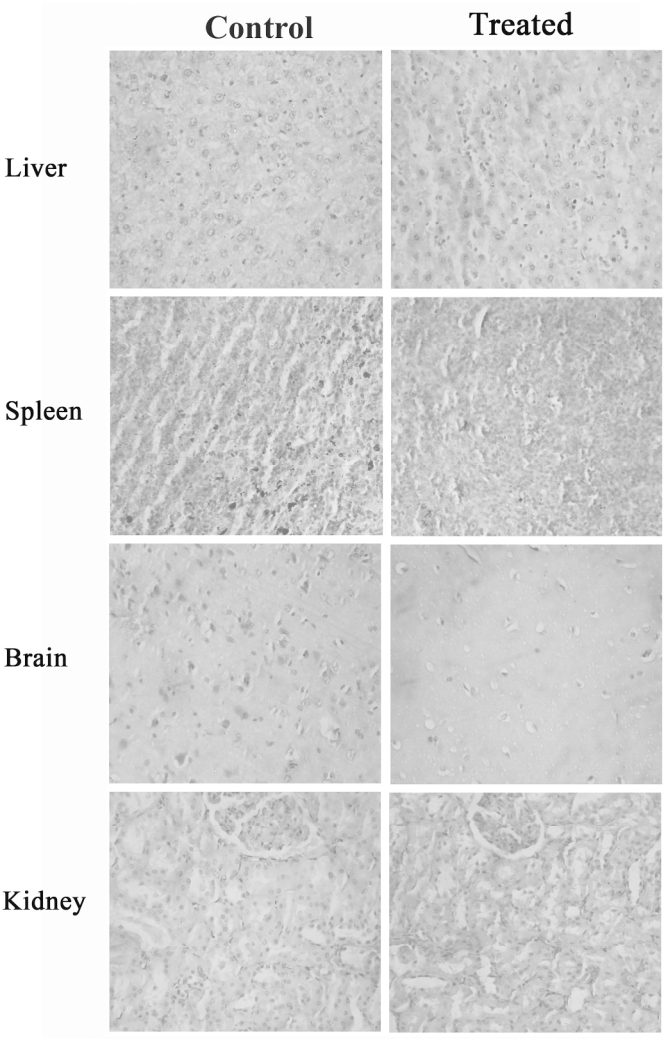
Table 2 Haematological and biochemical parameters of rats treated with CGM at 2.0 g/kg b.wt for 90 days

Table 3 Mutagenic study of *CGM* in the *Salmonella typhimurium* using reverse mutation assay with and without S9-mix

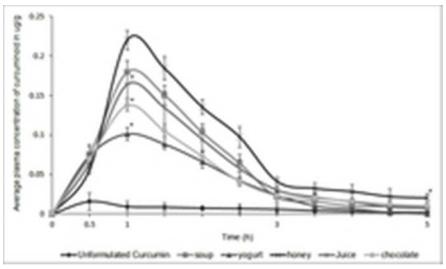
Table 4 Pharmacokinetics of curcumin CGM containing food consumption



Effect of subchronic administration of CGM on (A) body weight (B) food consumption (c) weight of organs of both male and female rats
212x215mm (300 x 300 DPI)



Histopathological analysis of rat organs treated with 2 g/kg b.wt. of CGM for 90 days
209x297mm (300 x 300 DPI)



Average concentration of curcumin observed in human plasma after consumption of various food items containing 100 mg of formulated curcumin, CGM, per serving. Serving sizes were 10g soup premix powder in 100 mL hot water, 20 g mango pulp in 100 mL water, 10 g honey, 20g chocolates and 30 g of yogurt. Standard curcumin was administered at 1000 mg dose as hard shell gelatin capsules (500 mg × 2). The data is expressed as the mean ± SD (n = 8); *p < 0.05; (100 mg CGM containing food versus 1000 mg standard curcumin)
18x10mm (300 x 300 DPI)

Table 1 Stability study of *CGM* performed as per in-house protocol prepared based on ICH guidelines. The samples were incubated at 40°C ± 2°C and 75% ± 5% RH (Accelerated stability study) for a period of 6 months.

Parameter	0 month	1 month	2 month	3 month	6 month
Appearance	Golden yellow to Orange colour granules	Golden yellow to Orange colour granules	Golden yellow to Orange colour granules	Golden yellow to Orange colour granules	Golden yellow to Orange colour granules
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Curcumin content (%)	40.8	40.6	39.9	40.5	40.2
Moisture (%)	2.3	2.3	2.3	2.3	2.2
Bulk density (g/mL)	0.51	0.51	0.51	0.51	0.51
Carbohydrate (g/100g)	80.04	80.14	79.26	80.71	80.16
Protein (g/100g)	6.0	6.1	5.8	6.0	6.0
Microbiology					
Total plate count	< 1000 cfu/g	300 cfu/g	400 cfu/g	350 cfu/g	350 cfu/g
Yeast & Mould	< 100 cfu/g	30 cfu/g	30 cfu/g	30 cfu/g	30 cfu/g
Coliforms	< 3 MPN/g	< 3 MPN/g	< 3 MPN/g	< 3 MPN/g	< 3 MPN/g
E.Coli	Absent/g	Absent/g	Absent/g	Absent/g	Absent/g
Salmonella	Absent/25g	Absent/25g	Absent/25g	Absent/25g	Absent/25g

Table 2. Hematological and biochemical parameters of rats treated with *CGM* at 2.0 g/kg b.wt for 90 days

Parameters	Control		2.0 g/kg		1.0 g/kg		0.5g/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
Biochemical Parameters								
ALT (U/L)	79.8 ±8.70	58.20±11.17	70.8±16.15	61.4±14.54	64.4±8.29	42.2±3.70	80.8±13.92	57.4±14.54
AST (U/L)	178.4±29.11	164.6±19.46	207 ± 42.64	157.2 ± 33.10	186.6 ± 29.24	161.8 ± 20.07	189.2 ± 18.73	185.8 ± 33.10
ALP (U/L)	351.4±27.22	325.0±42.52	376.4±26.81	266.2±52.49	327.6±51.26	231.2±36.77	360 ±34.32	262.2±52.49
Bilirubin (mg/dL)	0.5±0.07	0.40±0.07	0.54±0.05	0.54±0.09	0.5±0.07	0.5±0.07	0.5±0.10	0.48±0.09
Urea (mg/dL)	66.6 ± 8.26	34.8 ± 3.11	43.2 ± 3.90	40.6 ± 10.45	59.8 ± 11.69	44.0 ± 7.65	45.4 ± 7.65	38.8 ± 11.1
Creatinine (mg/dL)	0.70 ± 0.07	0.50 ± 0.23	0.72 ± 0.11	0.72 ± 0.08	0.70 ± 0.16	0.68 ± 0.19	0.60 ± 0.10	0.46 ± 0.13
Cholesterol (mg/dL)	73.4±7.44	77±17.39	65.2±3.56	84.4±6.47	63.2±6.38	82.8±5.26	78.2±12.79	69.0±2.83
Triglycerides (mg/dL)	97.8±12.5	127.8±11.92	88.2±5.17	107.4±12.10	86.0±5.79	98.8±18.24	109±11.11	118.4±11.55
HDL (mg/dL)	32.6±2.97	33.6±3.85	29.8±2.59	33.0±3.16	29.4±1.34	31.6±2.07	31.8±1.92	32.2±1.10
LDL (mg/dL)	21.2±8.98	18.2±11.67	17.8±3.35	30.0±6.89	16.6±4.56	31.4±8.20	24.4±10.74	13.2±3.11
VLDL (mg/dL)	19.6±2.61	25.6±2.51	17.6±1.14	21.4±2.41	16.8±1.79	19.8±3.63	20.0±3.16	24.0±2.45
Sodium (mEq/L)	145.36 ± 3.95	144.70 ± 2.72	143.12 ± 0.87	134.38 ± 2.17	141.98 ± 1.02	142.28 ± 4.03	146.26 ± 3.95	134.38 ± 1.09
Pottasium (mEq/L	7.27 ± 1.63	6.08 ± 1.27	5.89 ± 1.31	6.15 ± 1.30	4.68 ± 1.29	5.09 ± 1.13	5.08 ± 0.44	6.15 ± 1.44
Chloride (mEq/L)	103.86 ± 0.96	103.94 ± 1.22	104.50 ± 0.67	102.70 ± 2.48	103.38 ± 0.97	101.56 ± 2.87	104.24 ± 1.5	102.70 ± 2.54
Hematological parameters								
Hb (g/dL)	14.48 ± 0.94	13.16 ± 1.04	13.84 ± 1.22	13.62 ± 1.97	14.82 ± 1.35	14.52 ± 0.5	15.02 ± 0.58	14.26 ± 0.89
WBC ($\times 10^3/mm^3$)	9.5 ± 2.18	5.58 ± 1.54	7.84 ± 2.12	11.7 ± 4.23	12.28 ± 2.32	05.48 ± 1.96	10.26 ± 0.48	8.12 ± 3.21
Platelet ($\times 10^6/mm^3$)	514.2 ± 50	513.5 ± 69	579.6 ± 73	558.4 ± 91	468.4 ± 90	557.1 ± 69	502.3 ± 49	420.9 ± 87
RBC ($\times 10^6/mm^3$)	7.48 ± 0.40	6.48 ± 0.69	6.82 ± 0.47	6.96 ± 1.12	7.61 ± 0.67	7.07 ± 0.43	7.71 ± 0.18	6.98 ± 0.23
Neutrophils (%)	25.8±5.12	24.8±4.76	23.0±5.24	25.2±7.46	23.6±4.04	23.6±5.03	22.4±3.65	24.5±3.79
Lymphocytes (%)	64.6±4.83	65.8±5.76	68.4±4.93	65.6±8.38	67.0±3.54	66.8±5.72	69.6±4.77	68.3±2.99

Values are expressed as the mean ± SD, n = 5 animals of each sex per group.

Table 3 Mutagenic study of *CGM* with and without S9-mix in *Salmonella typhimurium* using reverse mutation assay.

	Doses of formulated curcumin (µg/plate)	**TA 98**	**TA 100**	**TA102**
(Without S9-mix)	Positive control[a]	861 ± 16	662 ± 16	860 ± 28
	Negative control[b]	33 ± 3	118 ± 6	121 ± 4
	5000 µg/plate	25 ± 3	96 ± 5	91 ± 12
	3000 µg/plate	28 ± 2	102 ± 4	103 ± 3
	1000 µg/plate	34 ± 1	110 ± 6	104 ± 4
	500 µg/plate	34 ± 2	125 ± 6	100 ± 4
	100 µg/plate	34 ± 1	124 ± 4	104 ± 5
(With S9-mix)	Positive control[c]	683 ± 16	884 ± 17	875 ± 25
	Negative control[b]	39 ± 5	129 ± 3	128 ± 4
	5000 µg/plate	35 ± 6	129 ± 9	104 ± 6
	3000 µg/plate	40 ± 6	146 ± 6	118 ± 4
	1000 µg/plate	44 ± 8	148 ± 10	109 ± 3
	500 µg/plate	36 ± 5	158 ± 8	124 ± 4
	100 µg/plate	38 ± 4	158 ± 13	127 ± 6

The values are mean ± standard deviation of 3 different determinations; [a] sodium azide (5 µg /plate) was used as mutagen; [b] spontaneous reversion (without mutagen and drug); [c]2-acetamidofluorene (20 µg/plate) was used as mutagen .

Table 4 Pharmacokinetic parameters calculated from plasma curcumin concentration – time plot for humans after CGM containing food consumption.

Sample administered	Subjects (n)	Dose	C_{max} (µg/g)	T_{max} (h)	$T_{1/2max}$ (h)	AUC_{0-6h} (µg/g)
Standard-Curcumin	8	1000 mg	0.015±0.005	0.5	0.7	205±34
soup	8	100 mg*	0.179±0.03	1	2.4	2460±78
yogurt	8	100 mg*	0.101±0.05	1	1.6	1435±96
honey	8	100 mg*	0.219±0.03	1	2.7	3075±61
Juice	8	100 mg*	0.163±0.07	1	2.4	2255±134
chocolate	8	100 mg*	0.137±0.04	1	2.0	1845±59
Cookies	8	100 mg*	ND$	ND$	ND$	ND$

* 100 mg *per* serving size of CGM was impregnated in each of the food item; 100 mg CGM contains 40 mg of curcumin; $ ND denotes 'Not detected'. C_{max} - maximum concentration of curcumin found in plasma, T_{max} - time at which maximum curcumin concentration was found in plasma, $T_{1/2max}$ - the time taken for 50% of absorbed curcumin to degrade