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Research Paper

Comparative bioavailability of curcuminoids from a water-dispersible high curcuminoid turmeric extract against a generic turmeric extract: a randomized, cross-over, comparative, pharmacokinetic study

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Abstract

Objectives The therapeutic utility of turmeric (*Curcuma longa* L., Zingiberaceae) is limited due to low bioavailability of its active principal curcuminoids. This study evaluates the pharmacokinetic characteristics of a natural, water-dispersible turmeric extract containing 60% curcuminoids (TurmXtra 60N), referred to as WDTE60N, compared to standard turmeric extract 95% (STE95).

Methods This open-label, two-way crossover, single oral dose, comparative pharmacokinetic study, randomized 14 subjects to receive one capsule of WDTE60N (150 mg curcuminoids) or three capsules of STE95 (500 mg curcuminoids each). The resulting dose ratio of actives for WDTE60N:STE95 was 1:10.

Key findings Peak plasma levels of free curcumin, total curcuminoids, tetrahydrocurcumin and demethoxycurcumin were similar ($P > 0.05$). C_{max} of total curcumin was higher ($P = 0.0253$) for WDTE60N at a 10-fold lower dose compared to STE95 (43.5 ± 28.5 vs. 21.3 ± 10.7 ng/ml). Mean AUC_{0-t} was higher ($P < 0.001$) for free curcumin and comparable for total curcumin and total curcuminoids with WDTE60N than with STE95. Five adverse events were reported in three subjects (mild in severity) and were unrelated to study products.

Conclusion WDTE60N showed higher absorption and comparable exposure for free curcumin, total curcumin and total curcuminoids at a 10-fold lower dose than STE95.

Keywords: bioavailability; curcumin; free curcumin; low dose; turmeric extract; Zingiberaceae

Introduction

Turmeric (*Curcuma longa* L., Zingiberaceae) has been a commonly used medicinal plant in Ayurveda, the traditional Indian medicinal system, for many centuries. Turmeric rhizomes contain hydrophobic polyphenols called curcuminoids, comprising curcumin, demethoxycurcumin and bisdemethoxycurcumin (Figure 1) responsible for the vibrant yellow colour of the turmeric root.[1–3] Of these, curcumin is the major curcuminoid and constitutes 77%

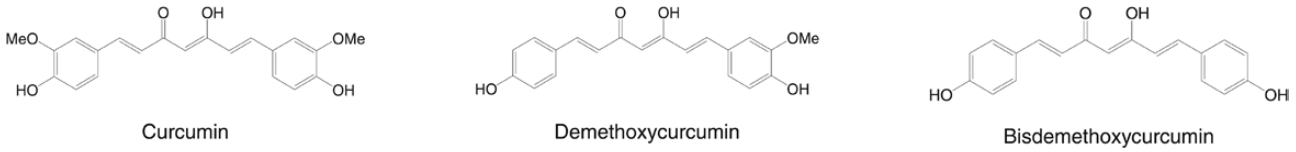

Figure 1 Structures of curcuminoids.

of the total curcuminoids. Curcuminoids exhibit a broad spectrum of pharmacological actions including antineoplastic, antidepressant, antioxidant, anti-inflammatory, immunoprotective, antimicrobial, antidiabetic and cognitive actions as demonstrated in several *in vitro*, preclinical, and clinical studies.[2, 4–11] Moreover, available evidence has shown that tetrahydrocurcumin (THC), a colourless or white metabolite of curcumin exhibits pharmacological activities similar to those of curcumin. Besides THC, other metabolites of curcumin have been identified, including the conjugates curcumin glucuronide and curcumin sulfate, which have been shown to be biologically inactive.[12–14] The mechanisms underlying the beneficial effects of curcumin are complex and involve several pathways including inhibition of pro-inflammatory factors; activation of peroxisome proliferator-activated receptor gamma cell signalling pathways; downregulation of IL-6, resistin, leptin and monocyte chemostatic protein; up-regulation of adiponectin and modulation of expression of host enzymes and other gene products.[2]

The clinical data regarding the pharmacokinetic (PK) characteristics and metabolism of curcuminoids are limited. Curcumin undergoes extensive phase I and II biotransformation. The double bonds of curcumin are subsequently reduced by reductase in enterocytes and hepatocytes to dihydrocurcumin, THC, hexahydrocurcumin and octahydrocurcumin.[13, 14] Curcuminoids are potent inhibitors of cytochrome P450 3A enzymes, UDP-glucuronosyltransferase enzymes and sulfotransferase metabolism.[15, 16] Although oral turmeric supplementation has shown efficacy in many clinical trials, its therapeutic utility is limited by poor absorption, fast metabolism and rapid elimination.[2] These factors, combined with its low aqueous solubility, result in low bioavailability of curcuminoids; therefore, a very large dose of approximately 6–8 g turmeric powder or 1500–2000 mg turmeric extract standardized to 95% is required to achieve therapeutic benefits. Such high dosage is shown to be associated with poor compliance, particularly when used long term. Moreover, studies also show that the incidence of adverse events (AEs) increases with increase in dose.[17] Therefore, there is an unmet need to develop a turmeric formulation that may provide therapeutic benefits at relatively lower doses. Efforts to enhance the bioavailability of curcuminoids are mainly focused on either blocking the curcumin metabolic pathway or improving absorption by changing the formulation to nanoparticles, emulsions, liposomes or cyclodextrin complexes.[18] Additionally, most bioavailable turmeric extracts available in the market are developed using synthetic excipients, and provide up to 20% of actives and these still need higher doses to achieve clinical efficacy. Thus, to overcome these limitations, and provide optimum benefit of the therapeutic effect of curcuminoids at a lower dose, a novel formulation of the natural, water-dispersible turmeric extract containing 60% natural curcuminoids (WDTE60N) was developed using a patented manufacturing technology. Also, the dose of 250 mg of test product containing 150 mg of curcuminoids was selected for the study based on the translational research, the results of the preclinical PK study conducted on Sprague Dawley rats (Supplementary Appendix 1).[19] Based on these results, a comparative PK study was designed to compare the PK of WDTE60N with that of the standard turmeric extract 95% (STE95) in healthy subjects.

Materials and Methods

Study design

This study was an open-label, randomized, balanced, two-treatment, two-sequence, two-period, two-way crossover, single oral dose, comparative PK study. The study was conducted at the pharmacological unit of an independent contract research organization, Enem Nostrum Remedies Pvt. Ltd, India as per the protocol approved by the Central Drugs Standard Control Organization–registered independent ethics committee (Ethos Ethics Committee, approval received on 19 December 2019), and in accordance with the pertinent requirements of the Declaration of Helsinki (Brazil, October 2013), Good Clinical Practices for Clinical Research in India 2005, New Drugs and Clinical Trials Rules 2019, ICH E6 (R2), Guidance on Good Clinical Practice and with all applicable requirements of Principles of Good Laboratory Practices (OECD and Schedule L-1 of D & C Rules 2019). All subjects were thoroughly informed about the study and signed informed consent forms before participating in the study.

Study population

Healthy adult men aged 18–45 years having a body mass index (BMI) ranging from 18.5 to 30.0 kg/m^2 with minimum bodyweight of 45 kg were enrolled in the study. Overall general health was ensured by performing clinical examination, medical history, haematology, serology, biochemistry assessments, electrocardiogram and chest X-ray. An important inclusion criterion was willingness to follow a diet free of turmeric, black pepper and any marketed spice mixture containing them starting at least 7 days before period I continuing till the end of period II. Key exclusion criteria were hypersensitivity to test or reference products and inactive ingredients thereof, history or presence of significant comorbidities, use of any product that could induce or inhibit hepatic microsomal enzyme before 1 month of first dosing and any history of drug and/or alcohol abuse in the past year. Eighteen subjects were enrolled as per the US FDA guidance document for bioequivalence studies that recommends a minimum of 12 evaluable subjects.

Study products and procedure

A block randomization schedule for two sequences (STE95 followed by WDTE60N and WDTE60N followed by STE95) was generated using PROC PLAN in SAS version 9.4 (SAS Institute Inc., USA) by an independent statistician. The pharmacist and principal investigator allocated the study products, stored in containers with appropriate labelling, to subjects as per the randomization schedule. The bioanalytical personnel were blinded to the assigned products.

Subjects were randomized to receive a single oral dose of one capsule of WDTE60N or three capsules of STE95. The WDTE60N capsule (Inventia Healthcare Ltd., India) contained 150 mg of

curcuminoids (curcumin, 132.6 mg; demethoxycurcumin, 31.7 mg; bisdemethoxycurcumin, 4.6 mg). The STE95 capsule contained 500 mg of curcuminoids (curcumin, 403.57 mg; demethoxycurcumin, 85.36 mg; bisdemethoxycurcumin 15.3 mg) and was formulated using the commercially available turmeric powder manufactured by Synthite Industries Pvt. Ltd, India. The resultant dose ratio of actives for WDTE60N to STE95 was 1:10.

All subjects were housed in the clinical pharmacology unit (CPU) from at least 60 h pre-dose to 24 h post-dose in both periods. Standardized meals, free of turmeric and black pepper and any marketed spice mixtures containing turmeric and/or black pepper were served during entire housing, including pre-dose and post-dose duration in both periods. As reported in the literature, piperine, which is an active constituent of black pepper, and a known inhibitor of hepatic and intestinal glucuronidation, was shown to enhance the oral bioavailability of curcumin by 2000% in humans.[20] Thus, to ensure that the study results were not affected by dietary interventions, all subjects were instructed to have all meals free of turmeric and black pepper and any marketed spice mixtures containing turmeric and/or black pepper (starting at least 7 days before period I till the end of period II) and all in-house meals were given free of turmeric and black pepper and any marketed spice mixtures containing turmeric and/or black pepper). For both periods, subjects received the assigned product with approximately 240 ml of water in the sitting posture under the supervision of an investigator/study staff who ensured that subjects had swallowed the study products. Subjects remained in a sitting or semi-recumbent posture and were not allowed to lie down for at least 2 h after administration of study products. Subjects were allowed to engage in normal activities while avoiding strenuous physical exertion during their entire stay in the CPU. A wash-out period of 7 days was maintained between the two treatment periods.

All subjects were monitored for occurrence of AEs and serious AEs throughout the study. Safety assessments included subject well-being assessment, clinical examination, haematology and biochemistry. Clinical examination (physical examination, systemic examination and vital signs including blood pressure, radial pulse rate, body temperature and respiratory rate) was carried out on the day of admission and before discharge in each period. Well-being and vital signs were measured before dosing and at 3, 6 and 11 h post-dose in each study period. Post-dose vital signs were assessed within ± 45 min of scheduled time. Subjects were not allowed to consume any medication including vitamins and herbal products during the period of 14 days before dosing in period I until 7 days after completion of period II. No subject was given concomitant therapy during the course of the study. Also, as curcuminoids are light sensitive,[21] all activities including dosing, blood sample collection, entire sample handling, processing, bioanalysis, accountability, dispensing and retention of investigational products were performed under yellow monochromatic light.

Sample collection and handling

Fourteen blood samples of 6 ml each were collected from each subject in each period. Pre-dose samples were collected at 12 and 2 h, and within 10 min before dosing. Post-dose samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 h. Direct venipuncture was used for sample collection at 12 h pre-dose while indwelling cannulation in a forearm vein was used for the remaining pre- and post-dose samples. Post-dose sampling was completed within 2 min from the scheduled sampling time. The blood samples were centrifuged at 4000 rpm at 4°C for 10 min within 60 min of collection and the resulting plasma samples were stored in duplicate at ≤70 ± 15°C until the analyses.

Sample preparation and chromatographic analysis

The plasma concentrations of total curcumin, demethoxycurcumin, bisdemethoxycurcumin, THC and total curcuminoids were estimated using validated liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) methods. Plasma samples were extracted by a protein precipitation method followed by enzymatic reaction using β-glucuronidase/arylsulfatase solution and final extraction was performed using a liquid-liquid extraction method using tert-Butyl methyl ether (TBME) as solvent. The solvent used for the extraction was evaporated under a stream of nitrogen and the residue was reconstituted with 0.15 ml of mobile phase before loading into an autosampler. Then, 10 µl was injected into the LC-MS/MS. All calibration curves and quality control samples were prepared in human plasma by spiking standard solutions. Correlation coefficients for all methods achieved R^2 values >0.990. Curcumin D6 (BioOrganics, Bengaluru, India) was used as internal standard for free curcumin, total curcumin and THC; and bisdemethoxycurcumin D8 (BioOrganics, Bengaluru, India) was used as internal standard for bisdemethoxycurcumin and demethoxycurcumin. Calibration curves were prepared for curcumin (VIVAN Life Sciences Pvt, Ltd., Mumbai, India), demethoxycurcumin (VIVAN Life Sciences), bisdemethoxycurcumin (SimSon Pharma Ltd., Mumbai, India) and THC (Simson Pharma).

Plasma concentration of free curcumin was estimated using separate validated LC-MS/MS method without using β-glucuronidase/arylsulfatase solution. Plasma samples were extracted by liquid-liquid extraction using TBME. The solvent used for the extraction was evaporated under a stream of nitrogen. The residue was reconstituted with 0.12 ml of mobile phase and then vortexed. The sample was transferred into auto-injector vials, and these were loaded into an autosampler. Then, 15 µl was injected into the LC-MS/MS.

Analysis of free and total curcumin and metabolites was carried out using an HPLC system coupled to a mass detector. The chromatographic separation of all compounds was achieved using C18 (100 × 4.6) mm; 3.5 µm column and mobile phase consisting of different ratios of acetonitrile and formic acid in water (0.005% w/v) at a flow rate of 0.5 ml/min. All analytes were detected using a mass detector equipped with turbo ion spray ionization source in a negative ionization mode. The analytes were detected in the multiple reaction monitoring mode. The mass spectrometer transitions (precursor/product) were as follows: curcumin (367.400/217.000), bisdemethoxycurcumin (307.200/187.200), demethoxycurcumin (337.200/217.100), THC (371.400/235.000), curcumin D6 (373.300/220.100) and bisdemethoxycurcumin D8 (315.200/191.100). The optimized parameters for the ionization source included spray voltage set at 4.5 kV, nebulizer gas flow rate of 35 units, turbo gas flow rate of 60–65 units, curtain gas flow rate of 22 units and temperature of turbo gas set at 600°C. The collision energies applied depended on the fragmentation behaviour of the molecule investigated. Using the optimized method, the obtained limits of quantification (LOQ) were as follows: free curcumin, 50.04 pg/ml; total curcumin, 5.00 ng/ml; bisdemethoxycurcumin, 0.20 ng/ml; demethoxycurcumin, 0.50 ng/ml and THC, 2.00 ng/ml. Each calibration curve was analysed individually by using least square weighted linear regression ($1/x^2$). Interday and intraday variability for each analyte were within the acceptance criteria.

PK assessments and statistical analysis

Pharmacokinetic parameters included maximum (peak) plasma drug concentration (C_{max}) and area under the plasma concentration–time curve from time zero to time t ($AUC_{0\text{-}t}$), time to reach maximum (peak) plasma concentration (T_{max}) and area under the curve (AUC)_% Extrap_obs (residual area) which were calculated by a noncompartmental model using SAS software Version 9.4 (SAS Institute Inc., Cary, NC, USA) for free curcumin, total curcumin, demethoxycurcumin, bisdemethoxycurcumin, THC and total curcuminoids. If the pre-dose plasma concentration of a given curcuminoid was >5% C_{max}, the subject was excluded from the statistical analysis; however, subjects continued for the PK assessments. The values for AUC_% Extrap_obs or $AUC_{0\text{-}t}$ were not reported for the cases who had not exhibited a terminal log-linear phase in the concentration versus time profile. All concentration values below the LOQ were set to zero for all PK and statistical calculations. Missing concentrations were not included in the PK and statistical analysis. The C_{max} and $AUC_{0\text{-}t}$ were reported as arithmetic means with their standard deviations, geometric least square means (GLSM) with its 90% confidence interval and intra-subject variability. Ratio of GLSM for test and reference was also calculated (T/R). Analysis of variance was performed for log-transformed PK parameters C_{max} and $AUC_{0\text{-}t}$ of test and reference formulations.

Table 1 Demographic characteristics of subjects (N = 14)

Characteristics	Mean ± SD	Coefficient of variation (%)	Range
Age (years)	33.5 ± 7.4	22.1	21–44
Weight (kg)	67.7 ± 6.9	10.2	59.9–82.6
Height (cm)	166.1 ± 4.9	2.9	157.3–177.7
BMI (kg/m^2)	24.6 ± 2.7	11.2	19.4–29.4

Results

From 30 December 2019 to 10 January 2020, 18 healthy subjects were enrolled in the study, of which 14 received products and completed the study (Supplementary Appendix 2). The mean age of the subjects was 33.5 ± 7.4 years and the mean BMI was 24.6 ± 2.7 kg/m^2 (Table 1). The PK data of curcuminoids were plotted on a plasma concentration versus time curve (Figure 2), and the calculated AUC, C_{max} and T_{max}, are given in Table 2.

The mean C_{max} values of free curcumin, total curcuminoids, THC and demethoxycurcumin were numerically higher for WDTE60N compared to STE95 but were not statistically significant ($P > 0.05$). Mean C_{max} of total curcumin was higher for WDTE60N at a 10-fold lower dose compared to STE95 ($P = 0.0253$). Similarly, the mean $AUC_{0\text{-}t}$ was comparable between the products for total curcumin and total curcuminoids ($P > 0.05$) but higher for free curcumin with WDTE60N compared to STE95 ($P < 0.001$).

After administering the WDTE60N formulation, the maximum plasma levels were achieved for free curcumin at 1.5 h; total curcumin, 3.0 h; total curcuminoids, 2.5 h and demethoxycurcumin, 2.0 h (Table 2). The GLSM, T/R ratio, relative absorption and relative bioavailability are summarized in Table 3. The data showed that the GLSM of C_{max} for the WDTE60N formulation was higher compared to STE95 for free curcumin (337.9 vs. 146.6), total curcumin (33.4 vs. 19.3), total curcuminoids (65.2 vs. 42.6), demethoxycurcumin (28.6 vs. 18.8) and THC (114.5 vs. 77.7); however, it was lower for bisdemethoxycurcumin (1.5 vs. 7.8).

There were five AEs reported in three subjects; all were mild in severity and were reported during the 7 days of post-study safety assessment. These AEs were not related to the study products.

Discussion

Turmeric is the most commonly studied medicinal plant with approximately 215 clinical trials reported in the clinicaltrials.gov database as of January 2020. The safety and therapeutic efficacy

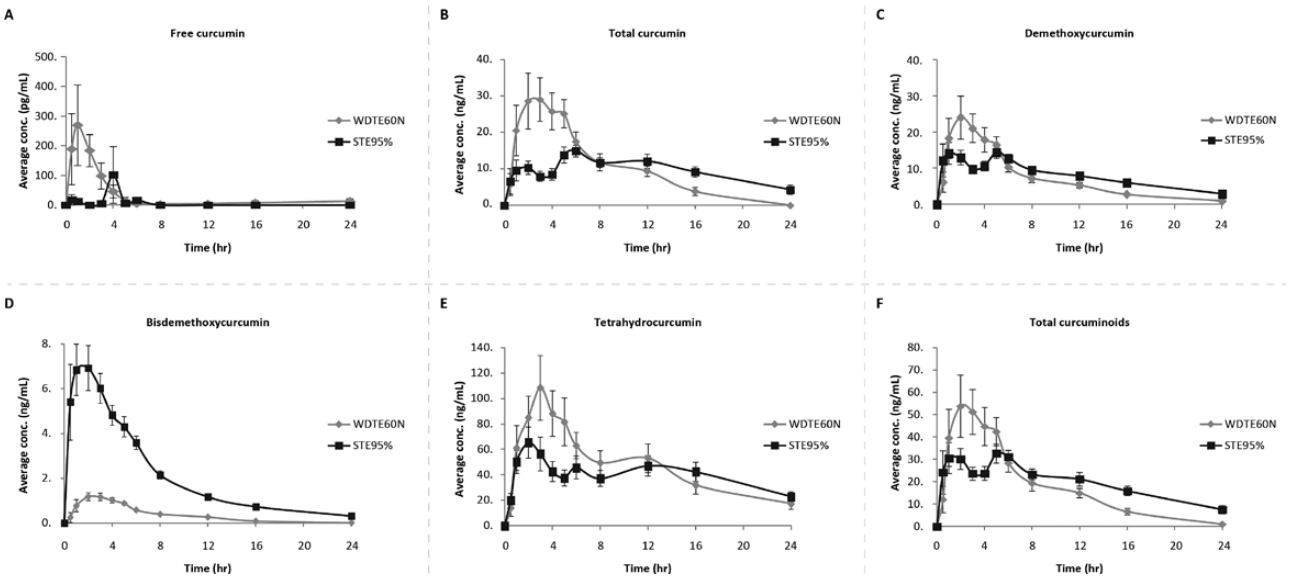

Figure 2 Mean plasma curcumin concentrations versus time plot – linear scale for water-dispersible turmeric extract formulation containing 60% natural curcuminoids and standard turmeric extract 95%. The error bars represent standard error of the mean. (A) Free curcumin concentration. (B) Total curcumin concentration. (C) Demethoxycurcumin concentration. (D) Bisdemethoxycurcumin concentration. (E) Tetrahydrocurcumin concentration. (F) Total curcuminoids concentration.

Table 2 Pharmacokinetic parameters of WDTE60N and STE95

	Pharmacokinetic parameters arithmetic mean ± SD (%CV)		
	C_{max} (ng/ml)	AUC_{0-t} (h.ng/ml)	T_{max} (h)[a]
Free curcumin[b] (*N* = 14)			
WDTE60N	438.9 ± 479.0 (109.2)	807.7 ± 676.1 (83.7)	1.5 (0.0–24.0)
STE95	154.0 ± 348.7 (226.5)	116.3 ± 209.9 (180.6)	1.0 (0.0–6.0)
P value*	0.0528	0.0003	
Total curcumin (*N* = 13)			
WDTE60N	43.5 ± 28.5 (65.6)	232.4 ± 152.9 (65.8)	3.0 (1.0–5.0)
STE95	21.3 ± 10.7 (50.5)	224.8 ± 87.4 (38.9)	5.0 (0.6–12.0)
P value*	0.0253	0.6869	
Demethoxycurcumin (*N* = 14)			
WDTE60N	36.0 ± 22.6 (62.6)	171.7 ± 92.6 (53.9)	2.0 (1.0–5.0)
STE95	21.4 ± 13.8 (64.4)	189.9 ± 55.9 (29.5)	2.0 (0.5–12.0)
P value*	0.0700	0.1691	
Bisdemethoxycurcumin (*N* = 14)			
WDTE60N	1.7 ± 0.9 (53.0)	8.2 ± 4.5 (54.6)	3.0 (1.0–5.0)
STE95	8.8 ± 5.2 (59.1)	51.6 ± 19.7 (38.1)	2.0 (0.5–3.0)
P value*	<0.0001	<0.0001	
Tetrahydrocurcumin (*N* = 14)			
WDTE60N	141.6 ± 88.7 (62.7)	1130.6 ± 743.5 (65.8)	2.5 (1.0–5.0)
STE95	87.7 ± 47.2 (53.8)	962.5 ± 523.1 (54.3)	2.5 (0.6–12.0)
P value*	0.0493	0.6823	
Total curcuminoids (*N* = 14)			
WDTE60N	82.4 ± 5 1.1 (62.0)	429.1 ± 253.6 (59.1)	2.5 (1.0–5.0)
STE95	47.9 ± 28.8 (60.13)	470.8 ± 151.2 (32.1)	2.0 (0.6–12.0)
P value*	0.0801	0.1866	

CV, coefficient of variation; SD, standard deviation; STE95, standard turmeric extract 95%; WDTE60N, water-dispersible turmeric extract formulation containing 60% natural curcuminoids.

[a]T_{max} reported as median (minimum–maximum).

[b]Free curcumin was measured in pg and remaining analytes were measured in ng.

**P* value derived from the analysis of variance test of log-transformed pharmacokinetic parameters.

Table 3 GLSM and its ratio for log transformed pharmacokinetic parameters

	GLSM and its ratio			ISCV (%) (90% CI)
	WDTE60N	STE95	T/R (%)	
Total curcuminoids (*N* = 14)				
C_{max} (ng/ml)	65.2	42.6	153.1	64.4 (102.9, 227.6)
AUC_{0-t} (h.ng/ml)	357.8	446.7	80.1	43.8 (60.4, 106.2)
Total curcumin (*N* = 13)				
C_{max} (ng/ml)	33.4	19.3	173.0	71.7 (109.7, 272.6)
AUC_{0-t} (h.ng/ml)	178.7	208.5	85.7	57.6 (58.7, 125.1)
Free curcumin (*N* = 14)				
C_{max} (pg/ml)	337.9	146.6	230.5	78.6 (117.5, 452.1)
AUC_{0-t} (h.pg/ml)	638.5	59.8	1068.5	68.4 (585.1, 1951.3)
Demethoxycurcumin (*N* = 14)				
C_{max} (ng/ml)	28.6	18.8	152.5	60.8 (104.5, 222.5)
AUC_{0-t} (h.ng/ml)	148.5	181.9	81.7	37.9 (63.8, 104.5)
Bisdemethoxycurcumin (*N* = 14)				
C_{max} (ng/ml)	1.5	7.8	19.2	29.3 (15.8, 23.2)
AUC_{0-t} (h.ng/ml)	7.1	48.2	14.7	32.7 (11.9, 18.2)
Tetrahydrocurcumin (*N* = 14)				
C_{max} (ng/ml)	114.5	77.7	147.5	49.7 (107.4, 202.4)
AUC_{0-t} (h.ng/ml)	894.2	834.2	107.2	46.0 (79.8, 144.0)

CI, confidence interval; ISCV, intrasubject coefficient of variation; LSM, least square means; STE95, standard turmeric extract 95%; WDTE60N, water-dispersible turmeric extract formulation containing 60% natural curcuminoids.

Calculation of relative absorption = (GLSM of test/dose of test) × (dose of reference/GLSM of reference) for the parameter C_{max}. calculation of relative bioavailability = (GLSM of test/dose of test) × (dose of reference/GLSM of reference) for the parameter AUC_{0-t}. For free curcumin, the LSM for test and reference products (except for C_{max} test) were not estimable due to the inability to meet the statistical calculation criteria of balanced designs. T/R is the ratio of GLSM for test and reference. As relative absorption or bioavailability is the normalized T/R ratio with respect to parameter C_{max} and AUC_{0-t}, respectively, which in mathematical terms is given by [exp]^(LSM[test] – LSM[Reference]) × (dose of reference/dose of test), the same was not calculable.

of curcuminoids is well documented for doses between 1500 and 2000 mg daily, but these high doses may impact the clinical benefits of long-term usage due to lack of compliance. Extensive efforts have been made to improve the bioavailability of turmeric extract (curcuminoids). These include nanoparticulate preparations, formulations with micelles, liposomes or gelatin, or as combinations with other herbal extracts and polysaccharide complexes of curcumin.[5, 22–24] However, only a few studies have systematically characterized the PK of curcuminoids for achieving comparable bioavailability with lower doses. In this study, we compared the PK characteristics of the WDTE60N formulation with those of STE95. While subjects received about 10-fold lower quantities of curcuminoids in WDTE60N compared to STE95, plasma-free curcumin, total curcumin and total curcuminoids were higher for WDTE60N. These results indicate that the water dispersibility of WDTE60N aids in absorption of the active moieties and provides high plasma levels of curcuminoids as compared to STE95. WDTE60N was formulated using a novel patented technology, which enhances its water dispersibility, and hence its bioavailability.

The majority of commercially available branded turmeric extract formulations contain about 20–40% curcuminoids, with the rest of the formulations being excipients, whereas WDTE60N contains 60% curcuminoids in its natural form. Though curcuminoids have been designated 'generally recognized as safe' (GRAS) by the US FDA, many of the marketed turmeric formulations use synthetic excipients that are commonly used in pharmaceuticals, the long-term safety of which may not be established. Thus, the safety of such nutraceutical turmeric formulations remains unknown, as these are usually self-administered by consumers for longer durations. Conversely, the use of natural diet-sourced excipients having GRAS status makes WDTE60N safe for long-term use. Also, the excipients used in WDTE60N are not reported to exert any effect on the metabolism of curcuminoids.

In this PK study, the T_{max} of WDTE60N was shorter than that of STE95 for total curcuminoids, so it may be assumed that its water dispersibility provides for rapid curcuminoid absorption. Moreover, we have observed comparable plasma levels of total curcuminoids from administration of a significantly lower dose compared to STE95. The geometric mean $AUC_{0\text{-}t}$ ratio of free curcumin was higher for WDTE60N. The geometric mean ratios of minor curcuminoids which are present in much lower percentages compared to curcumin, namely demethoxycurcumin and bisdemethoxycurcumin, were lower for WDTE60N compared to free curcumin (81.7 and 14.7 vs. 1068.5, respectively), indicating that these are either less well absorbed or more rapidly metabolized. It should be noted here that most pharmacological actions of turmeric are attributed to the plasma level of free curcumin.[25] Additionally, the available clinical evidence shows that the therapeutic benefits of turmeric formulations are attained by administration of curcuminoids.[26–28] Therefore, to gain maximum therapeutic benefit, it is prudent to select a turmeric formulation with established bioavailability of free curcumin as well as total curcuminoids.

Prior studies have shown 2–80-fold increases in the relative bioavailability of curcuminoids compared to either STE95 or other marketed turmeric formulations.[24, 29–31] In a study by Purpura *et al.*, the highest mean plasma concentrations of curcumin and total curcuminoids observed were 73.2 ± 17.5 ng/ml and 87.0 ± 20.5 ng/ml, respectively.[24] In another study by Asher *et al.*, the maximum curcumin concentration was found to increase with the phosphatidylcholine conjugate of curcumin compared to the standard curcumin (71 ng/ml vs. 57 ng/ml).[31] Relative bioavailability is calculated by adjusting (normalizing) the dose of test and reference formulations, and is rarely reflected in the dose selection for subsequent clinical efficacy studies of the same high-bioavailability turmeric ingredients.[32, 33] Relative bioavailability reported in the numerous turmeric PK studies is dose-adjusted bioavailability. It is unclear in some studies whether 'curcumin' denotes total/free curcumin or a combination of curcuminoids. Moreover, estimation of total curcumin without concurrent estimation of free curcumin does not provide a clear indication for the proportion of free curcumin and limits interpretation regarding bioavailability and potential efficacy of free bioactive curcumin. Some studies employed hydrolysis of blood samples before extraction and reporting results as 'curcumin' may overestimate the amount of curcumin and lead to misrepresentation of results. Conversely, the most important aspect of our study was the estimation of free curcumin without hydrolysing plasma samples before analysis. This provided an accurate estimation of free curcumin in plasma. Free curcumin estimation from the STE95 is reported in very few studies. In one study in healthy subjects, the mean peak concentration of free curcumin achieved from a 650-mg dose formulated curcumin product was 22.43 ng/ml, whereas plasma-free curcumin from dosing an equal quantity of STE95 was not detected.[34] Similarly, very few PK studies are reported using a low dose of STE95. The reason might be low to no detectable levels of curcumin from the standard curcumin products even when dosed at higher doses. For example, in an open-label, uncontrolled phase 1 pilot study of C3 complex with piperine, curcumin was not detectable before or after oral administration of 12 g of study drug at any timepoint.[35] In another study, in humans after a dose of 2 g curcumin alone, serum levels were either undetectable or very low.[20]

Under the given experimental conditions, free curcumin and total curcumin were more bioavailable in our study for WDTE60N compared to STE95. Our results form the basis of using a 10-fold lower dose of WDTE60N, which is equally bioavailable to a 95% turmeric formulation, in a prospective well-designed clinical efficacy/development program. However, the claims of other commercially available turmeric formulations for higher bioavailability are not supported by evidence of clinical efficacy at lower doses.

The dose of products tested was based on the results of preclinical study; dose-escalation studies should be performed for dose–response correlation in larger sample sets to establish whether increasing doses proportionately increase exposure of WDTE60N. Further studies are warranted to evaluate the relationship of plasma curcumin levels to biomarkers and clinical endpoints. Despite these limitations, this study is one of the few to have reported the PK of the majority of curcuminoids including free and total curcumin and total curcuminoids.[29, 36–40] As the AUC of WDTE60N is comparable to STE95 at a 10-fold lower dose, we anticipate that WDTE60N would be as effective as STE95 at this lower dose in our ongoing clinical efficacy studies. These clinical studies will help demonstrate the clinical benefits of a lower dose of WDTE60N for various therapeutic applications.

Conclusions

WDTE60N was found to have improved PK parameters and comparable exposure at a 10-fold lower dose to STE95. The degree of improvement in PK parameters was highest for free curcumin

and PK parameters were comparable for total curcumin and total curcuminoids. Both products were found to be safe and well tolerated after a single dose. Ongoing clinical efficacy and safety studies will help demonstrate the clinical relevance of a lower dose of WDTE60N and its safety and tolerability on long-term administration.

Supplementary Material

Supplementary data are available at *Journal of Pharmacy and Pharmacology* online.

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Author Contributions

S.T., R.S., K.V.A. and V.S. conceived the study. S.V. and V.U. conducted the study, undertook data collection and performed the data analyses. S.V. and V.U. interpreted the results with assistance from S.T., R.S. and K.V.A. S.T. prepared the first draft. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

S.T. and R.S. are employees of Inventia Healthcare Ltd. K.V.A. and V.S. are employees of Laila Nutraceuticals. S.V. and V.U. are employees of Enem Nostrum Pvt Ltd. The authors do not have any other conflicts of interest to declare.

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