*ejpmr, 2022,9(5), 372-380*

**SJIF Impact Factor 6.222**

**EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# COMPARATIVE BIOAVAILABILITY OF TURMERIC EXTRACT 65% + BLACK PEPPER EXTRACT 95%, TURMERIC EXTRACT 65% + BLACK PEPPER EXTRACT 40% AND TURMERIC EXTRACT 95% + BLACK PEPPER EXTRACT 95% CAPSULES OF BOTANIC HEALTHCARE PVT. LTD. VS TURMERIC EXTRACT 95% + BIOPERINE CAPSULES IN HEALTHY ADULT HUMAN SUBJECTS UNDER FASTING CONDITIONS

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**Article Received on 07/03/2022** **Article Revised on 28/03/2022** **Article Accepted on 19/04/2022**

## ABSTRACT

**Background:** Curcumin has a wide range of beneficial physiological and pharmacological activities, including antioxidant, anti-amyloid, anti-inflammatory, anti-microbial, anti-neoplastic, immune-modulating, metabolism regulating, anti-depressant, and neuroprotective and tissue protective effects. However, its poor solubility and poor absorption in the free form in the gastrointestinal tract and its rapid biotransformation to inactive metabolites greatly limit its utility as a health-promoting agent and dietary supplement. Recent advances in micro- and nano-formulations of curcumin with greatly enhanced absorption resulting in desirable blood levels of the active forms of curcumin now make it possible to address a wide range of potential applications, including pain management, and as tissue protective. Using these forms of highly bioavailable curcumin now enable a broad spectrum of appropriate studies to be conducted. A randomized, open label, analyst blind, balanced, four-treatment, four-arm, parallel, single dose, study was designed to evaluate the Comparative Bioavailability of Turmeric extract 65% + Black pepper extract 95%, Turmeric extract 65% + Black pepper extract 40% and Turmeric extract 95% + black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. Vs Turmeric extract 95% + bioperine capsules of Sabinsa Corporation. **Objectives:** To compare bioavailability of Turmeric extract 65% + Black pepper extract 95%, Turmeric extract 65% + Black pepper extract 40% and Turmeric extract 95% + black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. Vs Turmeric extract 95% + bioperine capsules in healthy adult human subjects under fasting conditions. **Conclusion:** The study concludes that the Test product-1 (T1): Turmeric extract 65% + Black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. Are Relative bioavailable to the Reference product, Test product-2 (T2): Turmeric extract 65% + Black pepper extract 40% capsules of Botanic Healthcare Pvt. Ltd. are Relative bioavailable to the Reference product, Test product 3 (T3): Turmeric extract 95% + black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. are Relative bioavailable to the Reference product; Turmeric extract 95% + bioperine capsules of Sabinsa Corporation.

**KEYWORDS:** Bioavailability, demethoxycurcumin, Curcumin solubility, absorption, Human study.

## INTRODUCTION

Turmeric (Curcuma Longa L.) is an age-old Asian Spice with more than 5000 years of history of usage in Indian traditional systems of medicine. An average of 1.5–2.5 g of turmeric was estimated to be consumed by Asians in their daily diet; which may correspond to about 60–100 mg of curcuminoids, the chief biologically active principle and yellow pigment of turmeric. Curcumin was first isolated from turmeric rhizomes in 1815 by the German scientists Vogel and Pelletier and its chemical structure was elucidated by Milobedeska and Lampe in 1910 as (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione . The first human clinical trial of curcumin was reported by Oppenheimer in 1937 for biliary disease and later its antibacterial property was identified by Schraufstatter and Bernt in 1949. The modern interest in curcumin was initiated with the early reports and human study by Kuttan et al. on its anti-cancer properties, and hypolipidemic effect. Since then, thousands of in vitro and in vivo studies have been reported on its pleiotropic mechanism of action and therapeutic potential against a wide range of disease conditions including cancer and Alzheimer's. There were about 120 clinical trials performed on curcumin by 2017

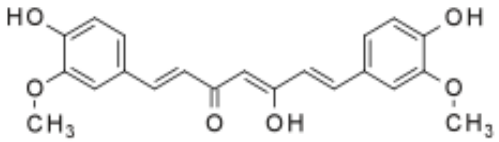
and out of which 17 double-blinded, placebo-controlled trials and 27 other trials have testified its safety and potential therapeutic benefits against various clinical conditions.

Industries have standardized solvent extraction techniques to produce 95 % pure curcuminoids from dried turmeric rhizomes, with a definite ratio of three polyphenolic molecules [curcumin or diferuloylmethane (72–80 %), demethoxycurcumin (DMC) (12–15 %) and bisdemethoxycurcumin (BDMC) (2–5%)], commonly referred to as 'curcumin'. Chemically, curcumin is an α, β-unsaturated diketone moiety with two phenolic groups. These functional groups makes the curcumin highly reactive, involving in proton donation and self-oxidation, reversible or irreversible nucleophilic addition (Michael reaction), hydrolysis, reductive degradations and enzymatic reactions. These chemical properties contributed to the multi-targeted mechanisms of action of curcumin through interaction with a wide range of membrane proteins, signaling molecules, free radicals and transcription factors. The structural features also contributed to the lability, insolubility, poor absorption, rapid biotransformation and fast elimination of curcumin from systemic circulation. Thus, curcumin can be considered as a class IV BCS molecule (Biopharmaceutics classification system) with interesting pharmacodynamics, but poor pharmacokinetics.

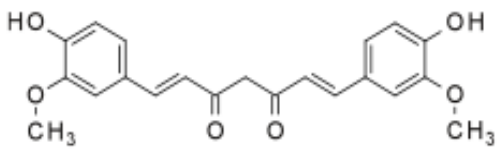
## DESCRIPTION

### Curcumin

Curcumin is a diarylheptanoid. IUPAC name is (1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-Dione. Its molecular formula is $C_{21}H_{20}O_6$ and molecular weight is 368.38. It is the principal curcuminoid of turmeric, which is a member of the ginger family (Zingiberaceae). Turmeric's other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The Curcuminoids are natural phenols that are responsible for the yellow color of turmeric. Curcumin can exist in several tautomeric forms, including a 1, 3-diketo form and two equivalent enol forms. The enol form is more energetically stable in the solid phase and in solution.




**Curcumin- Enol Form**




**Curcumin- keto Form**

## OBJECTIVES

### Primary Objective

To compared bioavailability of Turmeric extract 65% + Black pepper extract 95%, Turmeric extract 65% + Black pepper extract 40% and Turmeric extract 95% + black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. Vs Turmeric extract 95% + bioperine capsules in healthy adult human subjects under fasting conditions.

### Secondary Objective

To monitored and assessed safety and tolerability of the investigational products in healthy adult human subjects under fasting condition.

## METHODS

### Inclusion Criteria

Normal, healthy, adult, human subject of 18-55 years (both inclusive) of age, willing to give written informed consent, Willing to be available for the entire study period and to comply with protocol requirements**,** Body mass index in the range of 18 – 30 kg/m2 (both inclusive), Normal haemoglobin between 12.5 to 17.5 grams per deciliter (g/dL) for men, Normal health status as determined by baseline medical and medication history, at the time of screening and vital signs (blood pressure, pulse rate, and axillary temperature) measurements and physical examination at the time of screening as well as check-in during study period, With normal or clinically non-significant laboratory values as determined by hematological, biochemistry tests and urine analysis, With a negative test for Human Immunodeficiency Virus (HIV) type I/II antibodies or Hepatitis B surface antigen (HBsAg) or Hepatitis C virus antibodies, With a normal or clinically non-significant 12-lead ECG.

### Exclusion Criteria

Any medical or surgical conditions, which might significantly interfere with the functioning of the gastrointestinal tract and blood–forming organs, History of gastric or duodenal ulcer or GI bleeding or blood in stools anytime in the past, History of severe infection or major surgery in the past 6 months, History of Minor surgery or fracture within the past 3 months, Found Positive (+Ve) on Rapid antigen test for COVID-19 during screening, Haemoglobin level less than 12.5 grams per deciliter for men and 12 grams per deciliter for women, Any other clinical condition like diarrhea or vomiting within three days prior to dosing, Subjects who have been on an unusual or abnormal diet, for whatever reason, History or presence of significant gastric or duodenal ulceration, Use of any recreational drug or history of drug addiction.

### Ethics Committee Approval

All study related documents Protocol, Case Report Form, Dairy card, Investigator Brochure and Informed Consent Documents (English and Kannada Versions). Written Informed Consent was obtained from the subjects before the start of the trial and after due approval from

IEC/IRB. Ethics Committee notifications as per the GCP guidelines issued by Central Drugs Standard Control Organization and Ethical guidelines for biomedical research on human subjects issued by Indian council of Medical Research has been followed during the Conduct of the Study (ACE Independent Ethics Committee" IEC approval on 03 Aug 2021).

## STUDY OUTCOMES

### Primary Outcomes

$C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$
$T_{max}$, AUC_%Extrap_obs, λz and $t_{1/2}$

### Secondary Outcomes

$T_{max}$, AUC_%Extrap_obs, λz and $t_{1/2}$

### Disposition of Subjects

Thirty two (32) subjects has been enrolled in the study and, all thirty two (32) healthy male adult human subjects selected for participation and enrolled in the study as per the inclusion and/ or exclusion criteria. Thirty two (32) reported to the facility for study ICF presentation on 31 Aug 2021.

The study was planned on 32 subjects, i.e., Test product-1 (T1): Turmeric extract 65% + Black pepper extract 95% capsules of , Test product-2 (T2): Turmeric extract 65% + Black pepper extract 40% capsules, Test product 3 (T3) Turmeric extract 95% + black pepper extract 95% capsules and Reference product (R) Turmeric extract 95% + bioperine capsules.

### Detail procedure

The patients were screened and enrolled. The enrollment day was considered as the Day 0 i. e check in day followed by next day i.e. Day 1(Randomization, IP Dispensing, dosing and sample collection) day 2 will be check out.

Subjects was admitted and housed in the clinical facility from at least 10 hours before dosing and was checked out 24 hours after dosing. The subjects were received a standardized meal at about 4:00, 8:00, 12:00 and 24:00 hours after dosing.

Total 18 blood samples was collected from each subject during clinical study. The venous blood samples were withdrawn at pre-dose (00.000 hour, 7 mL) and at 00.250, 00.500, 00.750, 01.000, 01.500, 02.000, 02.500, 03.000, 03.500, 04.000, 05.000, 06.000, 08.000, 10.000, 12.000, 16.000 and 24.000 hours post-dose following drug administration.

After collection of blood samples from all the subjects at each time point, the samples were kept for centrifugation. The samples were centrifuged at 4000 RPM for 10 minutes at 2°C-8°C and documented.

The separated plasma sample were kept in equal quantity among the aliquots which transferred at a temperature of -70°C ±15°C in an appropriate container. The plasma samples will be handover for bioanalysis after completion of clinical phase.

Bio-analytical method was validated for Curcumin and Demethoxycurcumin in plasma using $K_2$EDTA as anti-coagulant for the sensitivity, specificity, linearity, accuracy and precision (repeatability and reproducibility), percent recovery and stability of samples (24 hours Interim stability, freeze-thaw stability, bench-top stability, auto sampler stability, short-term and long-term stability of stock solution of analyte and internal standard, long-term stability of analyte in matrix, stability of analyte in blood. Plasma samples was assayed for Curcumin and Demethoxycurcumin by using a validated HPLC/Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method.

### Statistical Analysis

Statistical Analysis of data obtained after the completion of study was analyzed using SAS software for windows, version 9.1, Schuirmann's two one-sided t-tests at 5% level of significance have been used to compare the average values of pharmacokinetic parameters determined after administration of test and reference products. Consistent with the Schuirmann's two one-sided t-tests for bioequivalence, 90% Confidence interval for the difference between treatments, least-squares means has been calculated for ln-transformed pharmacokinetic parameters i.e. $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ of Curcumin and Demethoxycurcumin.

## RESULTS

As per protocol Thirty two (32) healthy adult male subjects were enrolled in the study and all 32 subjects completed the study. Bio-analysis was performed on 32 subjects. Pharmacokinetic and Statistical analysis was performed for Test product-1 (T1) vs Reference product (R) on 13 subjects.

## PHARMACOKINETIC EVALUATION

Non-compartmental Analysis was applied for the estimation of Pharmacokinetic parameters $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ of $K_{el}$, and $t_{½}$ of Curcumin and Demethoxycurcumin, in plasma concentration time data SAS® software version 9.1.3 (SAS Institute Inc., CARY, USA).

The un-transformed mean pharmacokinetic parameters viz, $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ of $T_{max}$, $K_{el}$ and $t_{½}$ from plasma concentration time profile of Curcumin and Demethoxycurcumin for Test and Reference Product are tabulated as follows:

### Pharmacokinetic data summary tables and figures

Summary statistics of pharmacokinetic Parameters of Curcumin and Demethoxycurcumin after administration of Test (T) and Reference (R) products to healthy, adult, human male subjects under fasting conditions.

**Analyte: Curcumin (N=32)**
**T1 vs R**

| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 104.69 | 98.44 | 111.33 |
| $AUC_{0-t}$ | 97.88 | 91.13 | 105.14 |
| $AUC_{0-\infty}$ | 99.70 | 90.57 | 109.75 |

**T2 vs R**

| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 101.72 | 92.32 | 112.08 |
| $AUC_{0-t}$ | 100.38 | 91.90 | 109.65 |
| $AUC_{0-\infty}$ | 104.15 | 93.58 | 115.91 |

**T3 vs R**

| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 100.02 | 90.54 | 110.49 |
| $AUC_{0-t}$ | 94.10 | 86.66 | 102.18 |
| $AUC_{0-\infty}$ | 95.57 | 86.65 | 105.41 |

**Analyte: Demethoxycurcumin (32)**
**T1 vs R**

| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 99.54 | 90.86 | 109.06 |
| $AUC_{0-t}$ | 92.09 | 81.50 | 104.06 |
| $AUC_{0-\infty}$ | 95.65 | 85.47 | 107.04 |

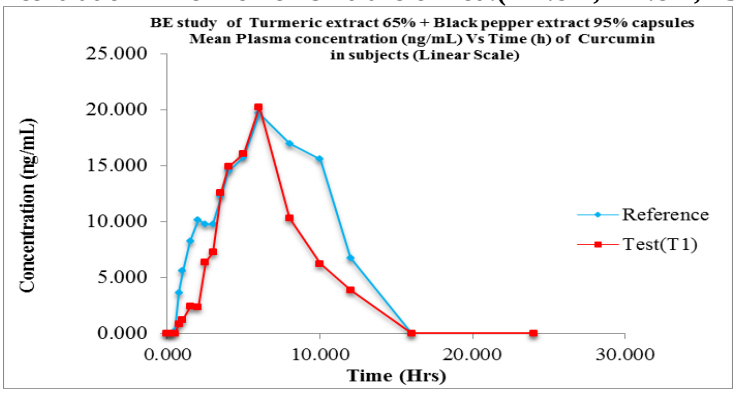
**T2 vs R**

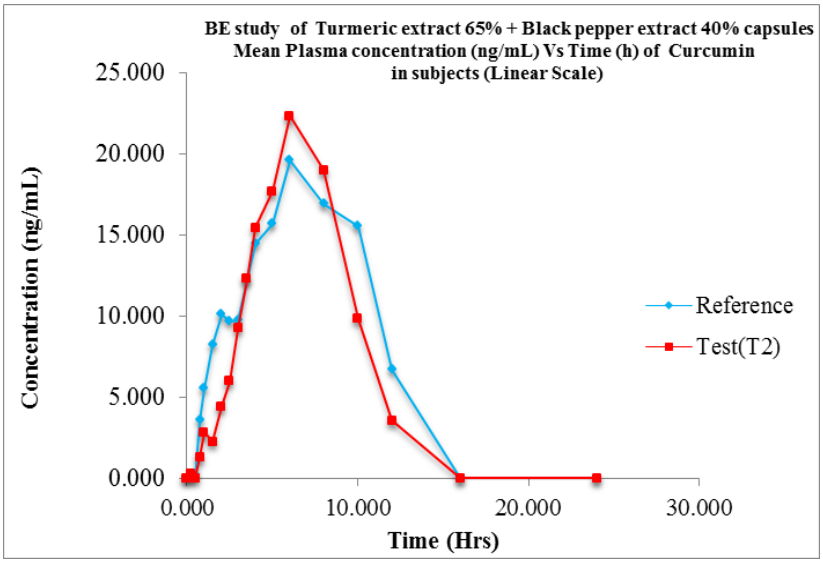
| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 108.30 | 97.60 | 120.17 |
| $AUC_{0-t}$ | 99.20 | 90.77 | 108.42 |
| $AUC_{0-\infty}$ | 106.23 | 95.23 | 118.51 |

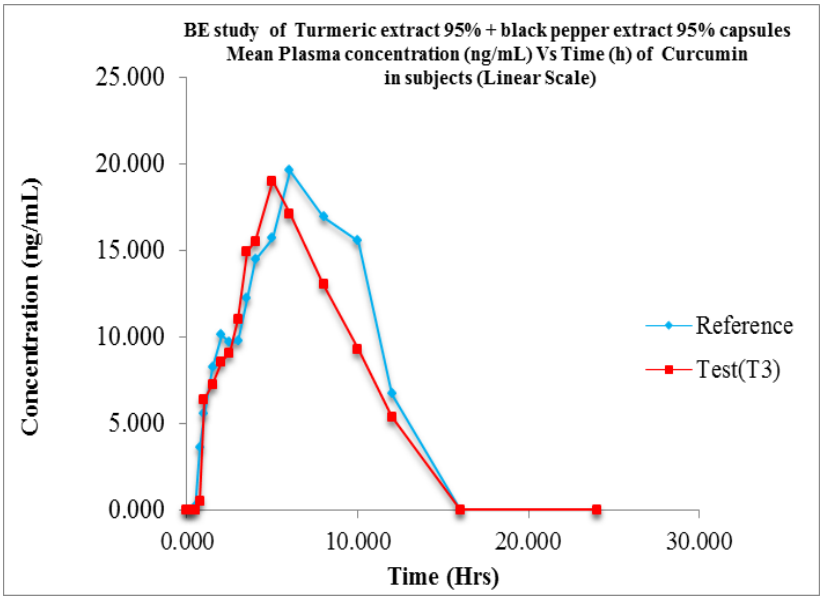
**T3 vs R**

| PK Parameters | Ratio % (Test/Ref) | 90 % Confidence Interval | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| $C_{max}$ | 103.48 | 94.15 | 113.75 |
| $AUC_{0-t}$ | 93.13 | 85.79 | 101.09 |
| $AUC_{0-\infty}$ | 92.92 | 84.06 | 102.70 |

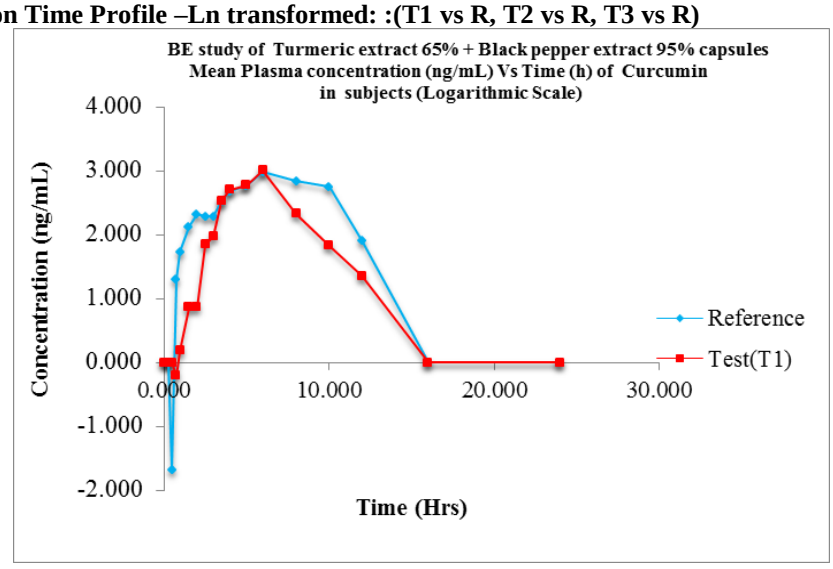
**Mean Concentration Time Profile –Un transformed and Ln transformed:**
**For Curcumin Mean Concentration Time Profile –Un transformed:(T1 vs R, T2 vs R, T3 vs R)**

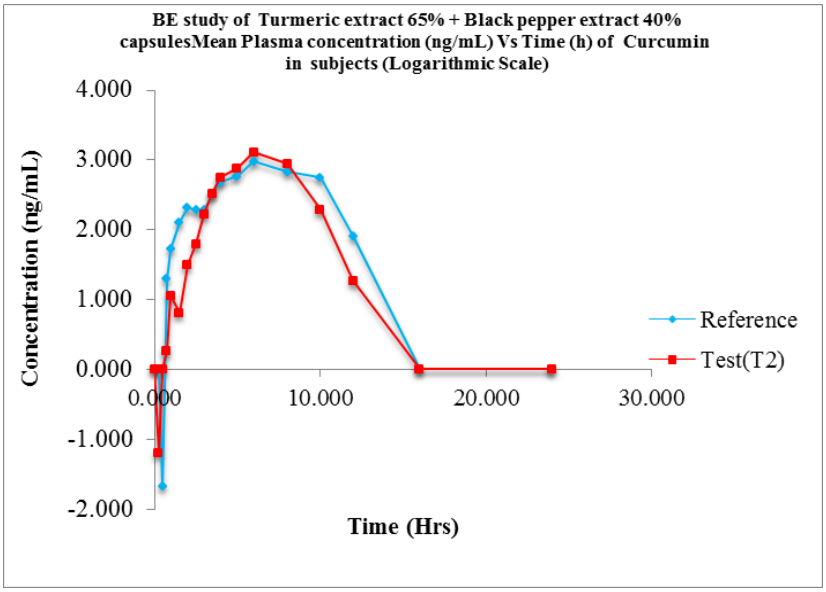


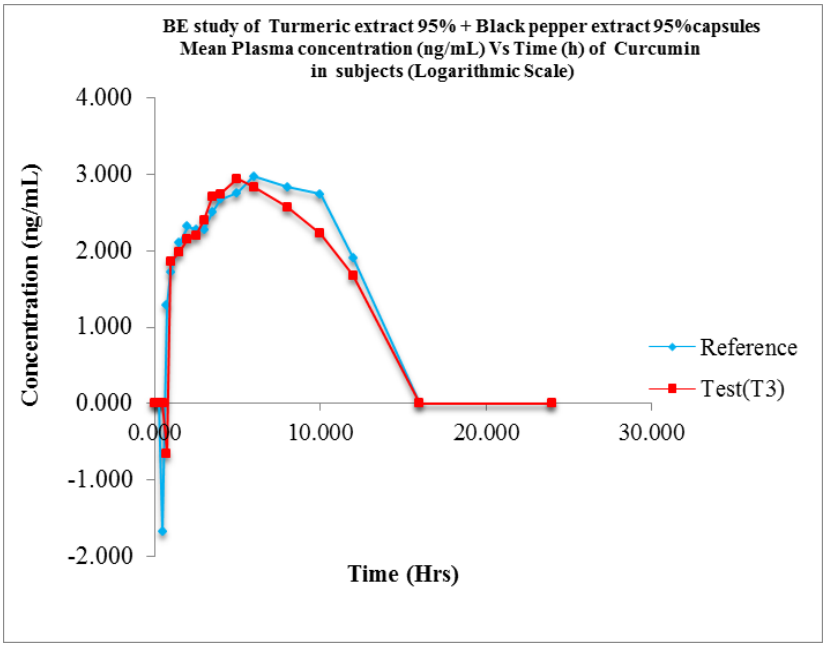



**Mean Concentration Time Profile –Ln transformed: :(T1 vs R, T2 vs R, T3 vs R)**

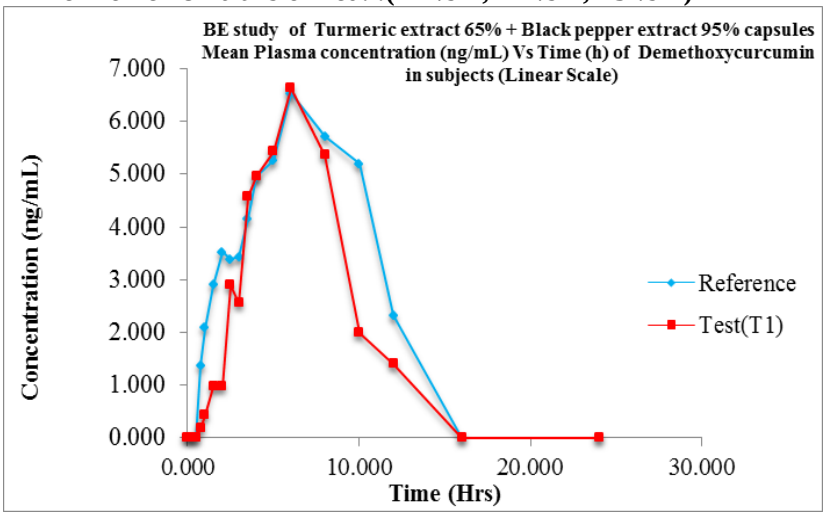


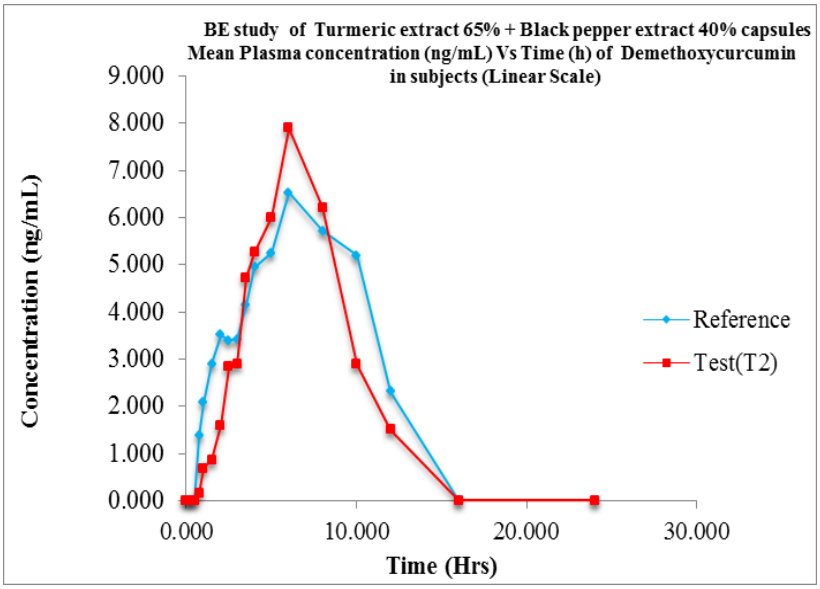


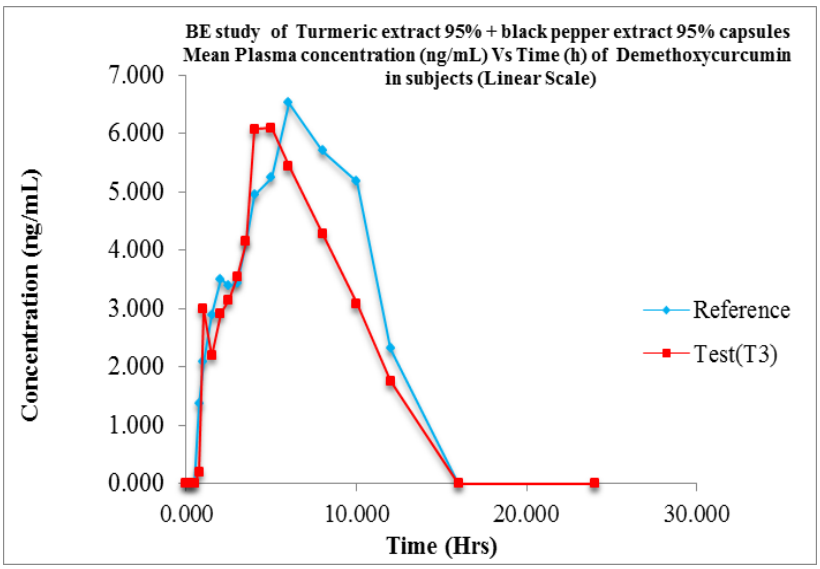




**For Demethoxycurcumin**

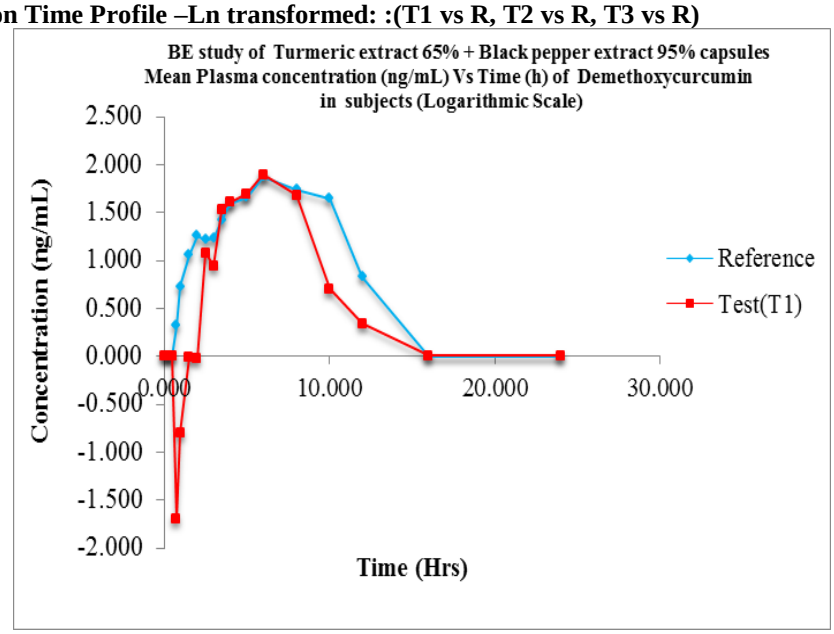
**Mean Concentration Time Profile –Un transformed: :(T1 vs R, T2 vs R, T3 vs R)**

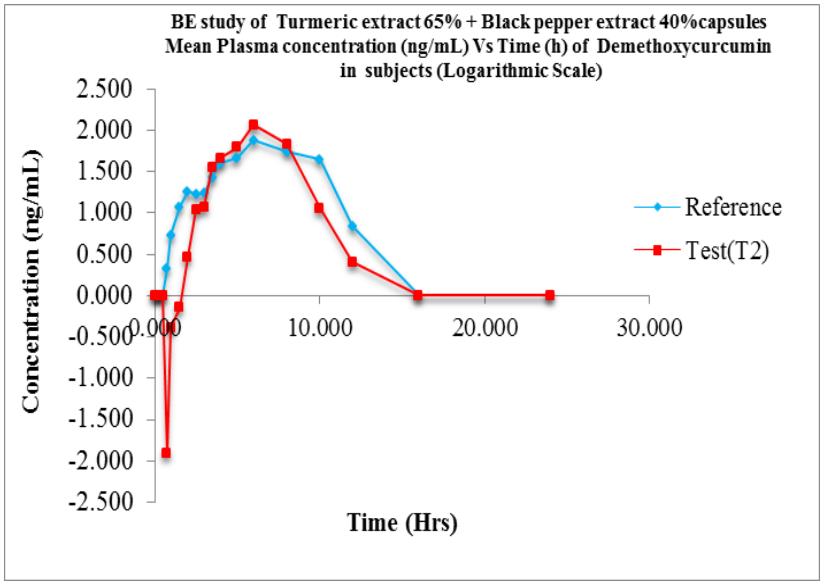


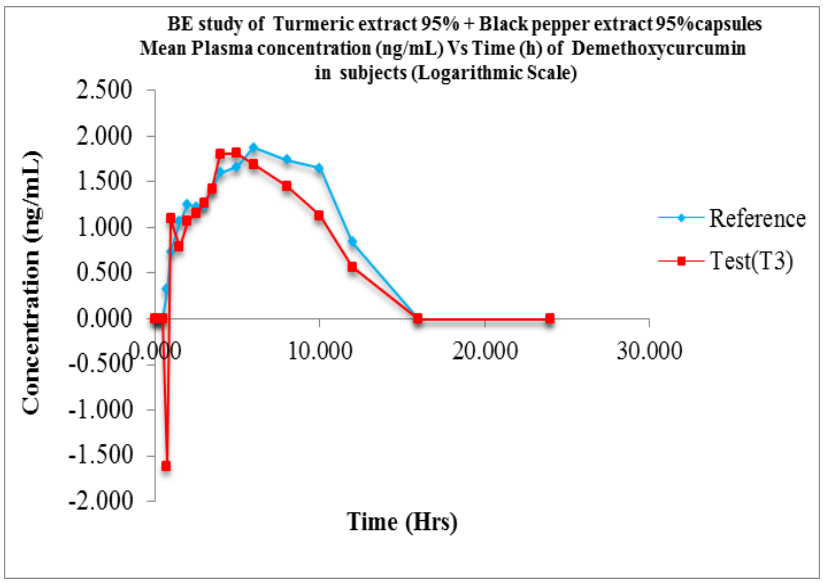



**Mean Concentration Time Profile –Ln transformed: :(T1 vs R, T2 vs R, T3 vs R)**






## DISCUSSION AND CONCLUSION

The Test product-1 (T1): Turmeric extract 65% + Black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. Are Relative bioavailable to the Reference product, Test product-2 (T2): Turmeric extract 65% + Black pepper extract 40% capsules of Botanic Healthcare Pvt. Ltd. are Relative bioavailable to the Reference product, Test product 3 (T3): Turmeric extract 95% + black pepper extract 95% capsules of Botanic Healthcare Pvt. Ltd. are Relative bioavailable to the Reference product.: Turmeric extract 95% + bioperine capsule in healthy adult male human subjects under fasting condition and was also well tolerated upon single dose administration.

Subjects were monitored for their well-being by recording their vital signs during, before and at the end of the study as per the protocol. Post study safety evaluation of all subjects was performed. All 32 subjects post safety sample was collected and the safety evaluations were found within normal limits for post safety analysis.

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