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Enhancement in Bioavailability of CurCousin®, a Minor Metabolite from *Curcuma longa* by Addition of BioPerine® —A Pharmacokinetic Study

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Abstract

In recent years, metabolic syndrome has been a growing health concern across the world. The role of nutraceuticals and functional foods in this area has a significant place due to the adverse effects of contemporary modes of treatment. CurCousin® is a nutritional ingredient containing bioactive Calebin A, (analog of Curcumin) with self-affirmed GRAS status. CurCousin® has been a clinically studied dietary supplement ingredient with a positive impact on body weight, lipid levels and metabolic health. Bioenhancers play an important role in increasing the bioavailability of the active in turn enhancing efficacy as well as reducing the dosage required to achieve the therapeutic effect. This study investigated the possible pharmacokinetic interaction between CurCousin® at two different doses (2.25 and 4.5 mg/kg) in the presence and absence of BioPerine® (0.27 mg/kg), a natural bioenhancer in Sprague-Dawley rats. The results revealed that the addition of BioPerine® into CurCousin® (2.25 mg/kg) half the dose when administered enhances the bioavailability and was equipotent to CurCousin® (4.5 mg/kg) double the dose without BioPerine®. Thus, leading to future clinical studies to evaluate its improved pharmacological efficacy as well as reduced therapeutic dosage.

Keywords

Metabolic Health, Bioavailability, Pharmacokinetics, CurCousin®, BioPerine®, Calebin A

1. Introduction

In recent years, there has been an increased concern about metabolic health due to faulty food habits, sedentary lifestyles, mental exhaustion, and stress. Metabolic syndrome refers to an aggregate of metabolic dysfunctions including insulin resistance, atherogenic dyslipidemia, central obesity, and hypertension, which is posing a significant health and social concern [1]. Metabolic syndrome has been widely reported to increase the risk of cardiovascular disease or type 2 diabetes mellitus. The data from the National Health and Nutrition Examination Survey (NHANES) reported a significant increase among young adults (age group 20 to 39 years) with the overall prevalence of metabolic syndrome at 34.5% [2]. Lifestyle changes along with medications are the general treatment approaches for metabolic syndrome. As there is no stand-alone treatment for metabolic syndrome and the conventional management demands the continued use of multiple drugs for a long duration, it has become challenging for patients. The use of bioactive compounds and functional foods may address the different aspects of metabolic syndrome like body weight, glucose metabolism, lipid profile, blood pressure etc. Recently, research on Calebin A (feruloylmethylferulate), a curcumin analog naturally present [3] in trace amounts in *Curcuma* species (*Curcuma longa*/ *caesia*) has been gaining attention. Calebin A belongs to a family of ferulate esters, rightfully called Calebenoids, which seem more stable than curcumin in physiological medium. The structural features of Calebin A have a keto and ester group whereas curcumin has a 1,3-diketonic structure (**Figure 1**) in the nearly fully enolic form [4].

Calebin A shows higher chemical stability in acidic and basic media in contrast to the noted instability of curcumin at higher pH values while sharing several physiological properties with curcumin [5]. Calebin A was shown to exhibit a variety of pharmacological activities like curcumin [6]. Calebin A has been proven for various health benefits such as metabolic disorders, obesity, inflammation, diabetes, dyslipidemia, osteoporosis, cancer, liver, and colon health [5] [7] [8].

CurCousin® is a patented nutritional ingredient containing 99% pure Calebin A with a self-affirmed GRAS status. CurCousin® has shown promising activity in managing the risk factors of metabolic syndrome both *in vitro* and *in vivo*. Clinical findings showed that CurCousin® (Calebin A) may be an effective supplement

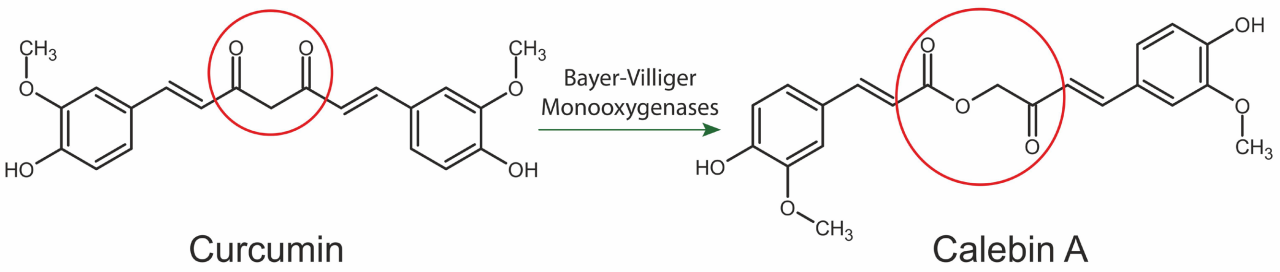

Figure 1. Chemical structural resemblances between Curcumin and Calebin A.

for managing abdominal obesity, dyslipidemia, and systemic inflammation in individuals with metabolic syndrome [9] [10]. A bioenhancer is an agent capable of enhancing the bioavailability and efficacy of a drug with which it is co-administered, without any pharmacological activity of its own at the therapeutic dose used [11] [12].

This study was undertaken to determine the effect of CurCousin® at two different doses (2.25 mg/kg and 4.5 mg/kg) in Sprague-Dawley rats and to evaluate whether the addition of natural bioenhancer, BioPerine® (0.27 mg/kg), a safe food component can increase the bioavailability at a lower dose making it almost equivalent to double dose. One such well-recognized natural bioenhancer in the market, backed by several clinical studies is BioPerine®. BioPerine® is prepared from the dried fruits of *Piper nigrum* (black pepper), standardized to 95% piperine with self-affirmed GRAS status. Multiple studies have been reported to confirm that BioPerine® significantly improves bioavailability when co-administered with various compounds through their increased absorption. Compounds such as curcumin, resveratrol, ginseng, β-carotene, coenzyme Q10, minerals (elemental iron, selenium), and vitamins (vitamin B6, vitamin C) were shown to enhance the bioavailability through increased absorption when administered with BioPerine® [13]. Thus, reducing the concentration of the dose will help to reduce the side effects and pricing of therapies that benefit more people due to their low cost.

2. Materials and Methods

2.1. Materials

The test materials, CurCousin® (Calebin A) and BioPerine® (*Piper nigrum* extract), used for the study were supplied by Sami-Sabinsa Group Limited. CurCousin® containing the bioactive Calebin A (analog of Curcumin), occurs only in trace amounts in the turmeric rhizomes. It was chemically synthesized with >99% purity by HPLC. BioPerine® prepared from the dried fruits of *Piper nigrum*, through a solvent extraction process was standardized to contain a minimum of 95% piperine by HPLC. CurCousin® has been clinically studied as a dietary supplement ingredient with a positive impact on body weight, lipid levels and metabolic health. With the addition of BioPerine®, it significantly enhances bioavailability through their increased absorption.

All other chemicals/reagents and solvents used throughout the experiment were of analytical grade.

2.2. Experimental Animals

The experimental protocol was initiated after obtaining the permission from the Institutional Animal Ethics Committee (IAEC), Karnataka, India. Female young adult Sprague-Dawley rats, with body weights ranging between 180 and 200 g, aged between 8 and 10 weeks were used in this study. The animals were housed in the animal facility of Sri Adichunchanagiri College of Pharmacy, Karnataka. All experimental procedures were carried out as per the Committee for the Pur-

pose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animals under standard animal house conditions. Throughout the study, the animals were kept in polypropylene cages, under standard laboratory conditions. The temperature was kept at 22˚C ± 3˚C with a relative humidity of 30% - 70%, and a 12-hour light/dark cycle with adequate fresh air supply (minimum of 12 - 16 air changes per hour). Daily temperature and relative humidity were recorded. Animals were kept in clean and sieved paddy husks. All animals were acclimatized to laboratory conditions for at least five days prior to the experiment. All animals had free access to food and water *ad libitum.*

2.3. Pharmacokinetic Interactions of CurCousin® with and without BioPerine® in Female Rats

The healthy young adult female animals were administered a single oral (gavage) dose of test material (CurCousin®) in the presence or absence of co-administration with BioPerine®. Before the test material administration, the animals were fasted overnight and allowed free access to water prior to the pharmacokinetic study. To perform pharmacokinetic studies, the experimental animals weighing 180-200 g were divided into two groups of different treatments and the groups were further subdivided into three subgroups of three animals each.

The animals were allotted by randomization and stratification to all the groups. Group I was administered as a standalone CurCousin® with a dose of 4.5 mg/kg while Group II was administrated with a single dose of 2.25 mg/kg CurCousin® along with 0.27 mg/kg BioPerine®, both orally. The grouping and respective doses are explained in Table 1.

Blood samples were collected from individual animals by orbital venous plexus in Eppendorf tube containing dipotassium ethylenediaminetetraacetic acid (K2-EDTA) as anticoagulant, at predetermined time intervals (0 min, 15 min, 30 min, 1, 2, 4, 12 and 24 h) after test material administration. The plasma was separated by centrifuging the blood samples at 3000 rpm for 10 minutes and immediately frozen at −80˚C until analysis.

Table 1. Grouping and dosage used in the study.

Test group + dose (human equivalent dose)	Test group + dose (rat equivalent dose)	Sub-groups	No. of animals	Blood sampling Time points (8)		
Group I CurCousin® (50 mg)	CurCousin® (4.5 mg/kg b.w)	Group 1	3	0 min	1 h	12 h
		Group 2	3	15 min	2 h	24 h
		Group 3	3	30 min	4 h	-
Group II CurCousin® + BioPerine® (25 mg + 3 mg)	CurCousin®+BioPerine® (2.25 mg/kg + 0.27 mg/kg b.w)	Group 1	3	0 min	1 h	12 h
		Group 2	3	15 min	2 h	24 h
		Group 3	3	30 min	4 h	-

The test materials were administered as a single dose through oral route by gavage to each animal, using gavaging needle fitted onto a disposable syringe of appropriate size. The body weight of individual animals was recorded at the beginning before the administration of the test item. Prior to dosing and during the study period, the animals were observed for clinical signs and pre-terminal deaths periodically at 15th min, 30th min, 1st h, 2nd h, 4th h, 12th h and 24th h. Cage-side observations like changes in the skin and fur, eyes, mucous membranes, also respiratory, circulatory, and autonomic and central nervous systems, motor activity, and behavior patterns of animals were observed and recorded. Observations were also directed at the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The animals were subjected to detailed veterinary examination prior to test item administration. Changes in skin and fur, eyes, mucous membrane, occurrence of secretions and excretions, autonomic activities, response to handling, changes in gait, posture, clonic or tonic movements, stereotypic and bizarre behaviors of animals were recorded. At the end of the experimental period, all the animals were sacrificed by carbon dioxide asphyxiation method using chamber and subjected to necropsy. External and internal gross pathological observations were recorded.

2.4. Instrumentation and LC-MS /MS Method

The LC-MS/MS analysis was performed using the LC-MS/MS instrument, Water ZEVO TQS analyst 4.1 version. The LC-MS/MS system consisted of an HPLC system and a tandem mass spectrometer with (+) ESI. The analytical column Acquity UPLC BEH (C18, 2.1 × 100) was used for chromatographic separation. The mobile phase was a mixture of 0.1% Formic Acid: Acetonitrile (Gradient). The injection volume was 10 µL. MRM transition for Calebin A: 385.09 > 176.83 (q1 to q2).

2.5. Preparation of Plasma and Serum Samples

The plasma samples for LC-MS/MS analysis were prepared by the protein precipitation method. At the time of analysis, plasma samples were removed from the deep freezer and thawed at room temperature. Sample preparation was carried out by adding plasma (50 µL) to 2 mL centrifuge tube and acetonitrile (150 µL) as precipitating agent. The samples were vortexed for 2 min and centrifuged at 4000 rpm for 7 min. After centrifugation, the supernatant layer was separated and was injected to the LC-MS/MS system for analysis.

2.6. Preparation of Calibration Curve

A calibration curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve should be generated for each analyte in the sample. A sufficient number of standards should be used to adequately define the relationship between concentration and response. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte.

The number of standards used in constructing a calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte/response relationship. Concentrations of standards should be chosen based on the concentration range expected in a particular study. A calibration curve should consist of a blank sample (matrix sample processed without internal standard/molecule of interest for analysis), and six to eight non-zero samples covering the expected range, including the lowest limit of quantification (LLOQ).

3. Pharmacokinetic Parameters

The pharmacokinetic parameters such as peak plasma concentration (C_{max}), time to peak concentration (T_{max}), and area under the plasma concentration-time curve ($AUC_{0\text{-}t}$), were calculated based on concentration over time by using pk1 and pk2 software. Area under the curve (AUC) is an important parameter in the field of pharmacokinetics and used commonly to assess the extent of exposure of a test material and its clearance rate from the body. The AUC (from zero to infinity) represents the total test material exposure with time in blood. In addition, AUC is useful for the therapeutic test material monitoring of test material with a narrow therapeutic index. The C_{max} is the maximum (or peak) serum concentration that a test material achieves in a specified compartment or test area of the body after the test material has been administered and T_{max} is the time required for a test material to reach C_{max}. For oral administration, C_{max} and T_{max} are dependent on the extent, the rate of drug absorption and the disposition profile of the test material. The C_{max} is often measured to show bioequivalence between a generic and innovator test material. According to the FDA, bioavailability and bioequivalence rely on pharmacokinetic measurements such as AUC and C_{max} which demonstrates systemic exposure of the test material. The elimination rate constant (K_{el}) describes the rate at which test material is removed from the body. We can calculate the half-life of test material from K_{el}, if the clearance of test material is very high, K_{el} is high resulting short half-life and vice versa.

4. Results

4.1. Clinical Observations

4.1.1. Body Weights

The body weight of individual animals was recorded at the beginning before administration of the test material. During the study period, no treatment related changes were observed in any of the groups, indicating the test material did not have any adverse effect on body weight. The summary of body weight measurements is depicted in Table 2(a).

4.1.2. Clinical Signs and Pre-Terminal Mortality

All the animals were observed for any clinical signs and pre-terminal deaths periodically at 15th min, 30th min, 1st h, 2nd h, 4th h, 12th h and 24th h following dosing. During the study period no-treatment related changes in clinical signs of

Table 2. **(a)**: Body weight of animals during initial and 24 hours after administration of test items (CurCousin® + BioPerine® and CurCousin®). **(b)**: Findings of clinical veterinary examination of individual female animals of both groups.

(a)

Test group + dose	Sub-groups	Weekly body weights (gm)	
		Initial	24 hours after test item administration
Group I **CurCousin® (4.5 mg/kg b.w)**	Group 1	200	197
		198	195
		192	189
	Group 2	194	191
		185	182
		188	185
	Group 3	184	181
		186	183
		190	187
Group II CurCousin® + BioPerine® (2.25 mg/kg + 0.27 mg/kg b.w)	Group 1	190	187
		185	183
		188	185
	Group 2	184	182
		186	183
		190	188
	Group 3	189	186
		192	188
		195	192

(b)

Test group	Group I CurCousin® (4.5 mg/kg)	Group II CurCousin® + BioPerine® (2.25 mg/kg + 0.27 mg/kg)
Skin and Fur	0	0
Eyes	0	0
Mucous membrane	0	0
Occurrence of secretions and excretions		
- Salivation	0	0
- Urine staining	0	0
- Fecal staining or diarrhea	0	0
- Nasal discharge	0	0
Autonomic activity		
- Lacrimation	0	0
- Piloerection	0	0
- Pupil size or pupillary	0	0
- Unusual respiratory	0	0

Continued

Response to handling	0	0
Changes in gait	0	0
Posture	0	0
Clonic or tonic movements	0	0
Stereotypic behavior		
- Repetitive circling	0	0
- Excessive grooming	0	0
Bizarre behavior		
- Self-mutilation	0	0
- Walking backwards	0	0

n = 3; "0": Absence of clinical sign.

toxicity, pre-terminal mortality and abnormal behaviors were observed in any of the groups.

4.1.3. Detailed Clinical Veterinary Examination

During the study period, the animals were subjected to detailed clinical veterinary examination (Table 2(b)) prior to test material administration and thereafter during the study. All the animals were found to be normal in appearance and healthy, no abnormal behavior was observed, including the changes in the skin and fur, eyes and mucous membranes, also respiratory, circulatory, autonomic and central nervous system, motor activity and behavior pattern. The findings were scored using the Irwin test. This test is an observational screening paradigm that is comprised of a sequence of tests used to assess the neurobiological and physiological state of animals. The parameters that are evaluated using the Irwin test include autonomic and sensorimotor functions, convulsive behaviour, and other activities produced by a test material after the administration. This test is used in pharmacokinetic and maximum tolerated dose preliminary studies to establish dose-response ranges for subsequent efficacy studies. Each parameter was scored on a scale 0 to 5, with scale 0 representing the response in animals that did not show any clinical signs of abnormality compared to normal animals and scale 5 representing a maximal impaired animal.

4.2. *In vivo* Pharmacokinetic Studies of CurCousin® after Oral Administration and Co-Administration with BioPerine®

The LC-MS/MS method was effectively used to analyse the chemical constituent after administration of CurCousin® in female Sprague-Dawley rats. LC-MS/MS chromatogram of the chemical constituent, Calebin A is shown in Figures 2(a)-(c). The concentration range for the calibration curve of Calebin A was 1 ng/mL to 50 ng/mL and r^2 was observed 0.99. The lower limit of quantification (LLOQ) was ~0.8 ng/mL (Figure 3).

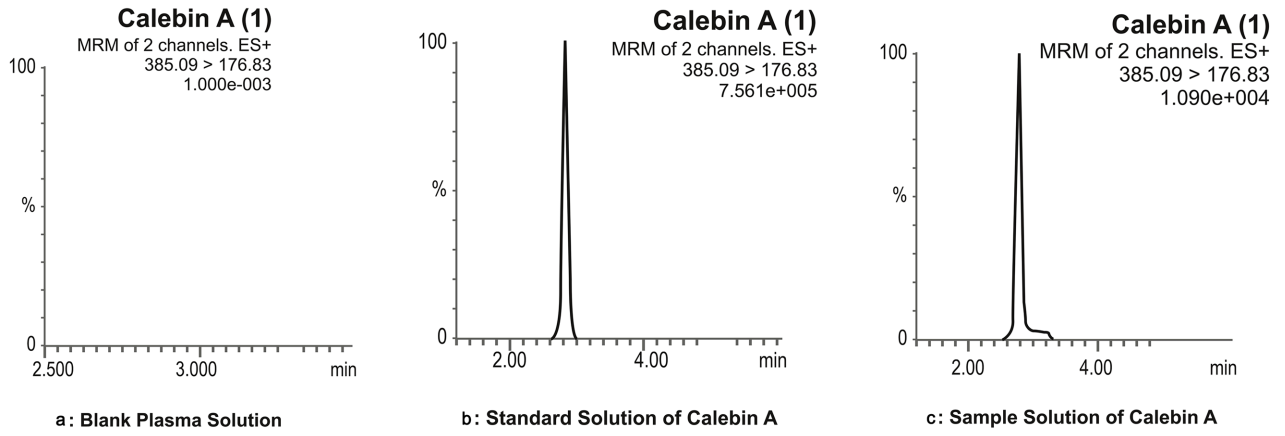

Figure 2. LC-MS/MS chromatogram of (a) Blank plasma solution (b) Standard solution and (c) Sample solution of Calebin A.

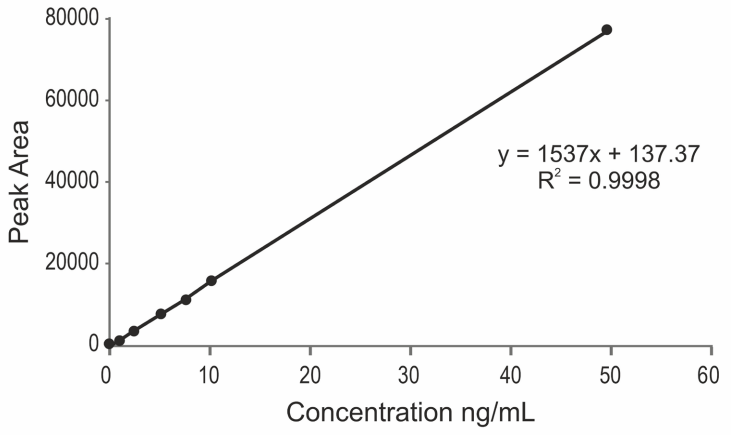

Figure 3. Calibration curve of Calebin A in rat plasma.

After oral administration of BioPerine®, the plasma concentration *versus* the time profile of CurCousin® are shown in **Figure 4.** The pharmacokinetic parameters of different groups are summarized in **Table 3**. As per the pharmacokinetic profile obtained, the administration of CurCousin® (4.5 mg/kg) showed the values of C_{max}, T_{max}, $AUC_{0\text{-}24h}$ and $t_{1/2}$ were 1.3496 ng/mL, 2 h, 19.0769 ng·h/mL and 24 h respectively. Similarly, the group treated with CurCousin® at a dose of 2.25 mg/kg along with the addition of BioPerine® at a dose of 0.27 mg/kg body weight in rats has the C_{max}, T_{max}, $AUC_{0\text{-}24h}$ and $t_{1/2}$ were 1.2293 ng/mL, 4 h, 18.5891 ng·h/mL and 20 h respectively as shown in **Table 3**.

The data demonstrated that $AUC_{0\text{-}24h}$ of CurCousin® at a lower dose of 2.25 mg/kg along with BioPerine® (0.27 mg/kg) was almost similar to double the dose of CurCousin® (4.5 mg/kg) without BioPerine®. Thus, the bioavailability of CurCousin® (2.25 mg/kg) with addition of BioPerine® (0.27 mg/kg) enhanced to 97.44% compared to stand-alone CurCousin® (4.5 mg/kg) which was almost similar. Overall, the data demonstrated that addition of BioPerine® (0.27 mg/kg) has enhanced the bioavailability of CurCousin® (2.25 mg/kg) equivalent to that of CurCousin® (4.5 mg/kg).

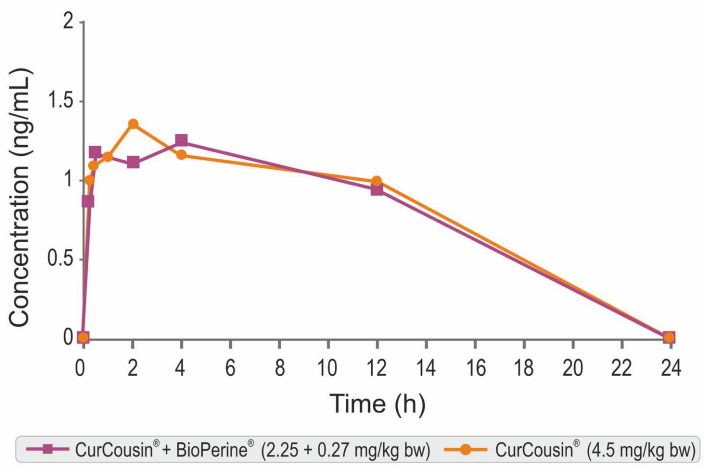

Figure 4. Plasma concentration (ng/mL) *vs.* time profile (0 - 24 hrs) of CurCousin® before and after administration of BioPerine®.

Table 3. Pharmacokinetic parameters of CurCousin® after oral administration in rats.

Pharmacokinetic parameters	Group I CurCousin® (4.5 mg/kg)	Group II CurCousin® + BioPerine® (2.25 + 0.27 mg/kg)	Bioavailability of Calebin A in CurCousin® (4.5 mg/kg) compared to CurCousin® + BioPerine® (2.25 + 0.27 mg/kg)
C_{max} (ng/mL)	1.3496	1.2293	97.44%
T_{max} (h)	2	4	
K_{el} (h)	0.0286	0.0350	
$t_{1/2}$ (h)	24.2434	19.7917	
$AUC_{0\text{-}24}$ (ng·h/mL)	19.0769	18.5891	
$AUC_{0\text{-}\infty}$ (ng·h/mL)	53.3480	45.1119	

C_{oax}: Maximum observed concentration; T_{max}: Maximum observed time; K_{el}: Elimination rate constant; $t_{1/2}$: Half-life; AUC: Area under the curve; $AUC_{0\text{-}24}$: Area under the curve 0 - 24 h; $AUC_{0\text{-}\infty}$: Area under the curve zero to infinity. Compared statistically: CurCousin® (Group 1) *vs.* CurCousin® + BioPerine® (Group 2) p values: C_{max} 0.213; $AUC_{0\text{-}24}$ 0.465; $AUC_{0\text{-}\infty}$ 0.923.

4. Discussion

In the modern era, lifestyle changes have brought a major setback in metabolic health. Metabolic syndrome has become a serious health concern across the world. The role of nutraceuticals emerges to be highly crucial due to the potential side-effects of contemporary mode of treatment. Bioenhancers are compounds that help to enhance the bioavailability of the active substance through increased absorption by enhancing the efficacy as well as reducing the dosage required to achieve the therapeutic effect. In this study, BioPerine®, a natural bioenhancer has been used, which has been proven to significantly improve the bioavailability when co-administered with various compounds such as curcumin, resveratrol, ginseng, β-carotene [14], coenzyme Q10 [15], minerals (elemental iron, selenium), and vitamins (vitamin B6, vitamin C) [13]. The bioavailability enhancement mechanism of piperine could be elucidated through

the-modulation of P-glycoprotein-mediated drug/ nutrient efflux, interaction with metabolic enzymes involved in biotransformation of drugs/nutrients, enhanced intestinal absorption, and its thermogenic action [13]. CurCousin® is a nutritional ingredient containing 99% pure Calebin A, a curcumin analog with potent pharmacological benefits has been clinically proven to have positive impact in managing healthy weight, lipid levels and metabolic health [9] [10]. The study was conducted in female Sprague-Dawley rats to determine the pharmacokinetic profile of two different doses of CurCousin® *i.e.* 2.25 mg/kg and 4.5 mg/kg and to evaluate whether the addition of BioPerine® (0.27 mg/kg) can enhance the bioavailability at a lower dose making it almost equivalent to double dose. Oral administration revealed no significant change in body weight, no clinical signs of toxicity, pre-terminal mortality and abnormal behaviors in any of the groups and all the animals were found to be healthy. After administration of test material at different time intervals the blood samples were collected from individual animals by orbital-venous plexus to determine the pharmacokinetic parameters.

The study concluded that the $AUC_{0\text{-}24h}$ of CurCousin® (2.25 mg/kg) administered with BioPerine® (0.27 mg/kg) had 97.44% increase which is almost equal to CurCousin® (4.5 mg/kg), inferring that CurCousin® half the dose when administered with BioPerine® was equipotent to CurCousin® double the dose without BioPerine®. Overall, this study demonstrated that, addition of BioPerine® enhanced the bioavailability of CurCousin®, thereby paving way for future clinical studies on CurCousin® with BioPerine® to investigate its improved pharmacological efficacy and reduced dosage of active making more consumer friendly.

5. Conclusion

In the present study, the results of the pharmacokinetic investigation revealed that co-administration of BioPerine® significantly improved the pharmacokinetic parameters of CurCousin®. The study demonstrated that the addition of BioPerine® can enhance the bioavailability and bioefficacy of CurCousin® by reducing the effective dose of CurCousin® from 4.5 mg/kg bw to half the dose of 2.25 mg/kg bw.

Conflict of Interest

The authors have no conflict of interest to disclose.

References

[1] Fahed, G., Aoun, L., Bou Zerdan, M., Allam, S., Bou Zerdan, M., Bouferraa, Y. and Assi, H.I. (2022) Metabolic Syndrome: Updates on Pathophysiology and Management in 2021. *International Journal of Molecular Sciences*, **23**, Article 786. https://doi.org/10.3390/ijms23020786

[2] Hirode, G. and Wong, R.J. (2020) Trends in the Prevalence of Metabolic Syndrome in the United States, 2011-2016. *JAMA*, **323**, 2526-2528. https://doi.org/10.1001/jama.2020.4501

[3] Zeng, Y., Qiu, F., Takahashi, K., Liang, J., Qu, G. and Yao, X. (2007) New Sesquiterpenes and Calebin Derivatives from *Curcuma longa*. *Chemical and Pharmaceutical Bulletin*, **55**, 940-943. https://doi.org/10.1248/cpb.55.940

[4] Goenka, S., Nagabhushanam, K., Majeed, M. and Simon, S.R. (2019) Calebin-A, A Curcuminoid Analog Inhibits *α*-MSH Induced Melanogenesis in B16F10 Mouse Melanoma Cells. *Cosmetics*, **6**, Article 51. https://doi.org/10.3390/cosmetics6030051

[5] Brockmueller, A., Mueller, A.L., Kunnumakkara, A.B., Aggarwal, B.B. and Shakibaei, M. (2022) Multifunctionality of Calebin A in Inflammation, Chronic Diseases and Cancer. *Frontiers in Oncology*, **12**, Article 962066. https://doi.org/10.3389/fonc.2022.962066

[6] Oliveira, A.L., Martinez, S.E., Nagabushnam, K., Majeed, M., Alrushaid, S., Sayre, C.L. and Davies, N.M. (2015) Calebin A: Analytical Development for Pharmacokinetics Study, Elucidation of Pharmacological Activities and Content Analysis of Natural Health Products. *The Journal of Pharmacy and Pharmaceutical Sciences*, **18**, 494-514. https://doi.org/10.18433/J32310

[7] Lai, C.S., Liao, S.N., Tsai, M.L., Kalyanam, N., Majeed, M., Majeed, A. and Pan, M.H. (2015) Calebin-A Inhibits Adipogenesis and Hepatic Steatosis in High-Fat Diet-Induced Obesity via Activation of AMPK Signaling. *Molecular Nutrition & Food Research*, **59**, 1883-1895. https://doi.org/10.1002/mnfr.201400809

[8] Tyagi, A.K., Prasad, S., Majeed, M. and Aggarwal, B.B. (2016) Calebin A Down-regulates Osteoclastogenesis through Suppression of RANKL Signalling. *Archives of Biochemistry and Biophysics*, **593**, 80-89. https://doi.org/10.1016/j.abb.2016.02.013

[9] Majeed, M., Majeed, A., Pandey, A., Lad, P.S. and Vuppala, K.K. (2016) Efficacy and Tolerability of a Novel Formulation for Weight Management in Obese Subjects: A Randomized, Double Blind, Placebo Controlled, Clinical Study. *International Journal of Ayurveda and Pharma Research*, **4**, 10-17.

[10] Majeed, M., Nagabhushanam, K., Devarajan, T.V., Saklecha, S., Reddy, S.V.K. and Mundkur, L. (2023) A Minor Metabolite from *Curcuma longa* Effective against Metabolic Syndrome: Results from a Randomized, Double-Blind, Placebo-Controlled Clinical Study. *Food & Function*, **14**, 4722-4733. https://doi.org/10.1039/D2FO03627D

[11] Randhawa, G.K., Kullar, J.S. and Rajkumar. (2011) Bioenhancers from Mother Nature and Their Applicability in Modern Medicine. *International Journal of Applied and Basic Medical Research*, **1**, 5-10. https://doi.org/10.4103/2229-516X.81972

[12] Kesarwani, K., Gupta, R. and Mukerjee, A. (2013) Bioavailability Enhancers of Herbal Origin: An Overview. *Asian Pacific Journal of Tropical Biomedicine*, **3**, 253-266. https://doi.org/10.1016/S2221-1691(13)60060-X

[13] Majeed, M., Nagabhushanam, K., Mundkur, L., Thazhathidath, S., Paschapur, M. and Manoharan, T.S. (2017) BioPerine®—Nature's Own Supernutrient, Bioavailability Enhancer and Thermonutrient. NutriScience Publishers LLC, New Jersey.

[14] Badmaev, V., Majeed, M. and Norkus, P.E. (1999) Piperine, An Alkaloid Derived from Black Pepper Increases Serum Response of Beta-Carotene during 14-Days of Oral Beta-Carotene Supplementation. *Nutrition Research*, **19**, 381-388. https://doi.org/10.1016/S0271-5317(99)00007-X

[15] Badmaev, V., Majeed, M. and Prakash, L. (2000) Piperine Derived from Black Pepper Increases the Plasma Levels of Coenzyme Q10 Following Oral Supplementation. *The Journal of Nutritional Biochemistry*, **11**, 109-113. https://doi.org/10.1016/S0955-2863(99)00074-1