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Bioavailability Studies of BioTurmin-WD (Water Dispersible Curcuminoids) Using Caco-2 Cell Model

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Abstract

Curcuminoids is the mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin obtained from dried rhizomes of *Curcuma longa,* commonly used for its wide therapeutic value. However, the absorption efficacy of curcuminoids is too low to exhibit its proper therapeutic value. Thus, a new preparation named as BioTurmin-WD (water dispersible curcuminoids) was developed for improved bioavailability. By using human Caco-2 cell monolayer, the permeability efficacy of BioTurmin-WD was evaluated and compared with that of 95% pure curcuminoids. Caco-2 model predicts the *in vivo* absorption of drugs across the gut wall by measuring the rate of transport of a compound across the Caco-2 cell line. BioTurmin-WD was added to the apical layer and basolateral samples were collected over 120 min to examine the concentration diffusing across the cell monolayer. Permeable curcuminoids across the cell monolayer was analysed through reverse phase high pressure liquid chromatography (RP-HPLC). Apparent permeabilities (P_{app}) of BioTurmin-WD and 95% curcuminoids were found to be 5.89×10^{-6} and 2.65×10^{-6} cm/s respectively. The apparent permeability coefficient of BioTurmin-WD was 7.03-fold higher than 95% pure curcuminoids. Percentage permeability of BioTurmin-WD (0.2945) was much higher than 95% curcuminoids (0.0859). Results indicated that BioTurmin-WD have a much higher absorption capacity (bioavailability) compared to 95% pure curcuminoids. Thus, BioTurmin-WD may be useful as a dietary supplement with greater bioavailability to exert clinical benefits in humans at a lower dosage.

Keywords: BioTurmin-WD, bioavailability, Caco-2 cells, *Curcuma longa*, curcuminoids, permeability

1. Introduction

Curcumin, demethoxycurcumin and bisdemethoxycurcumin are altogether known as curcuminoids, obtained from dried rhizomes of *Curcuma longa*. In Ayurvedic system of medicine, turmeric is commonly known for its medicinal values such as antiseptic, wound healing and anti-inflammatory properties (Kulkarni, Maske, Budre, & Mahajan, 2012). Turmeric is widely used to treat biliary disorders, sinusitis, rheumatism, hepatic disorders and diabetic complications. The yellow pigment of turmeric is being consumed as a dietary spice since ancient times and is a major ingredient of curry powders in India. Research on turmeric indicated that curcumin inhibits platelet aggregation, reduces blood cholesterol, suppresses symptoms associated with type 2 diabetes, and prevents low density lipoprotein oxidation and rheumatoid arthritis (Sasaki et al., 2011; Belcaro et al., 2010). In addition, curcumin has been reported to exhibit antibacterial, antioxidant and antimicrobial activities (Gupta, Patchva, & Aggarwal, 2013). The U.S. Food and Drug Administration has approved curcumin as a "generally recognized as safe" substance and may be used as a food additive (U.S. FDA., 2005). In several countries, curcumin is being used as a supplement. For example: turmeric has been used in preparation of cosmetic ingredient in India; in Japan and Korea, curcumin is served in drinks; in China, used as a colorant; in Malaysia, used as an antiseptic; in the United States, turmeric is used as a preservative and coloring agent (Gupta, Patchva, & Aggarwal, 2012).

Though curcumin is therapeutically effective against many ailments, but one of the major problems with curcuminoids is its poor bioavailability (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Bioavailability is defined as the rate and extent to which the active ingredient is absorbed and becomes available at the site of action (U.S. FDA., 2003). Absorption of drugs is dependent on aqueous solubility and intestinal permeability

(Burton, Goodwin, Vidmar, & Amore, 2002). The efforts had grown in parallel with the pharmaceutical industry to improve oral drug bioavailability. The new strategies have been required to develop orally active therapeutics drugs for better bioavailability (Gomez-Orellana, 2005).

The efforts had been put together to develop the curcuminoids formulation for better bioavailability. A water dispersible curcuminoids formulation was developed and named it as BioTurmin-WD. The permeability efficacy or bioavailability of BioTurmin-WD was compared with 95% pure curcuminoids through Caco-2 cell line (Human, colon adenocarcinoma cell line). Caco-2 cell is used to determine the absorption potentials of drug substances, their transport and metabolism mechanisms (Ming et al., 2004; Constanze et al., 2000). Based on the above context bioavailability of the developed BioTurmin-WD was carried out through permeability in Caco-2 cell monolayer.

2. Materials and Methodology

2.1 Materials

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), trypsin, penicillin, streptomycin, amphotericin B, ringer solution, Hank's balanced salt solution (HBSS) were purchased from Sigma Aldrich Co, St Louis, USA. Dimethyl sulfoxide (DMSO), propanol, HPLC grade acetonitrile and water were procured from E. Merck Ltd., Mumbai, India. 96 microwell plates were obtained from Tarsons Products Pvt. Ltd. (Kolkata, India). 12 thincert wells was procured from Greiner bio- one (Germany) and used for permeability study.

2.2 Sample Preparation

Turmeric rhizomes were obtained from cultivated source (Gundulupet, Chamarajanagar, Mysore, Karnataka, India). 95% pure curcuminoids were manufactured as per the standard operating procedure (SOP) of Olive Lifesciences, Tumkur, Karnataka, India. 30% water dispersible curcuminoids (BioTurmin-WD) was formulated from 95% curcuminoids extract at Research and Development Center, Olive Lifesciences, Tumkur, Karnataka, India.

2.3 Cell line Preparation

Caco-2 cells of human origin were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) at 95% humidity and 37 ºC in an atmosphere of 5% CO_2, supplemented with 10% FBS, penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml). The stock cultures were grown in 25 cm^2 culture flasks. Confluent monolayer cultures were dissociated with Trypsin Phosphate Versene Glucose (TPVG) solution [0.2% trypsin, 0.02% Ethylenediaminetetraacetic acid (EDTA), 0.05% glucose in PBS]. Regular media changes were carried out. Cells were used for experiments 28 days after seeding.

2.4 Cytotoxicity Study Through 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay

Cytotoxic dose was selected based on MTT assay (Francis, & Rita, 1986). In brief, monolayer cell was trypsinized. Cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added to 96 well microtitre plates. After 24 h, when a partial monolayer was formed, the supernatant was flicked off. Monolayer was washed. Different concentrations of test solution were added on to the partial monolayer in microtitre plates. The plates were incubated at 37 ºC for 3 days in 5% CO_2 atmosphere. Microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, test drug solutions were discarded and 50 µl of MTT solution prepared in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 ºC in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader (BioTek, Winooski, USA) at 540 nm. Concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Growth Inhibition} = 100 - [(\text{Mean absorbance of individual test} / \text{ Mean absorbance of control}) \times 100]$$

2.5 Membrane Permeability Study

Membrane permeability study with Caco-2 cells was performed with slight modification (Bozdağ-Pehlivan et al., 2011). Briefly, monolayer cultures of Caco-2 were trypsinized. 6×10^4 cells were seeded per cm^2 into the thin layer of thincert wells (Greiner bio-one, Germany) with 0.4 µm mean pore size and growth area of 1.1 cm^2 (12-well). Transport buffer (ringer solution) was added to apical and basolateral parts and incubated at 37 ºC for

30 min to attain equilibrium. BioTurmin-WD and 95% pure curcuminoids were dissolved in Hank's balanced salt solution (HBSS). Pre warmed test item combinations in transport buffer (500 µl) were added to the apical side of the thincerts. 1500 µl of transport buffer was added to the basolateral side of each thincert. The plate was incubated at 37 °C with 5% CO_2 humidity incubator. 100 µl samples were collected from basolateral side and fresh transport buffer of equal volume was replaced at different time intervals i.e., 15 min, 30 min, 60 min and 120 min. The collected samples were stored at -20 ºC for further RP-HPLC analysis to determine percentage permeability.

2.6 Reverse Phase- High Performance Liquid Chromatography (RP-HPLC) Analysis

Curcuminoids concentration in the collected samples was determined by RP-HPLC analysis. The RP-HPLC system (Shimadzu, Kyoto, Japan) consisting of two LC – 20AP controller pump; SIL – 10AP autosampler with 20 µl loop; SPD-M20A PDA detector and integrated LC solution software. Separation was achieved using Phenomenex ODS2 (250 × 4.6 mm; 5 µm) column (USA). Separation was achieved with a two pump gradient program for pump A: water and pump B: acetonitrile. Solvents were filtered through a 0.45 µm filter membrane prior to use. Mobile phase was pumped through the column with a flow rate of 2 mL/min. The analytes were analyzed at 429 nm. Quantity of permeable curcuminoids in collected samples was determined by means of a calibration curve (Kratz et al., 2011).

2.7 Determination of Permeability Coefficient

The apparent permeability coefficient (P_{app}, cm/s) was determined according to the following equation: $P_{app} = (dQ/dt)*1/AC_o$, where dQ/dt is the slope of the cumulative concentration of the compound in the receiving chamber over time (steady-state flux; mol/sec), C_o is the initial concentration applied in apical side (mol/ml) and A is the surface area of the porous membrane (A = 1.13 cm^2) (Kowapradit et al., 2010).

2.8 Statistical Analysis

Experimental data were expressed as mean ± standard error of mean (SEM) for individual sample. Statistical analyses were performed using GraphPad InStat Version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

The permeability study of water dispersible curcuminoids was evaluated through Caco-2 cell monolayer. The dose of the test substance was confirmed based on the cytotoxicity study using MTT assay against Caco-2 cells. The test samples were applied to the apical side of a Caco-2 cell monolayer and incubated for 2 h to assess the permeability of curcuminoids. The cytotoxity and permeability results are follows:

3.1 Cytotoxicity Study

Two fold serial dilution ranges from 100 – 6.25 µg/ml were optimized to determine the cytotoxic concentration (CTC_{50}). The percentage growth inhibition of test samples with the concentration ranges from 100-6.25 µg/ml was depicted in Figure 1. The CTC_{50} value is generated from the dose response curve. The experimental CTC_{50} value of BioTurmin-WD and 95% curcuminoids on Caco-2 cells was found to be 38.01 ± 0.1 and >50 µg/ml respectively. Based on this CTC_{50} value, test dose for permeability study was confirmed to 20 µg/ml.

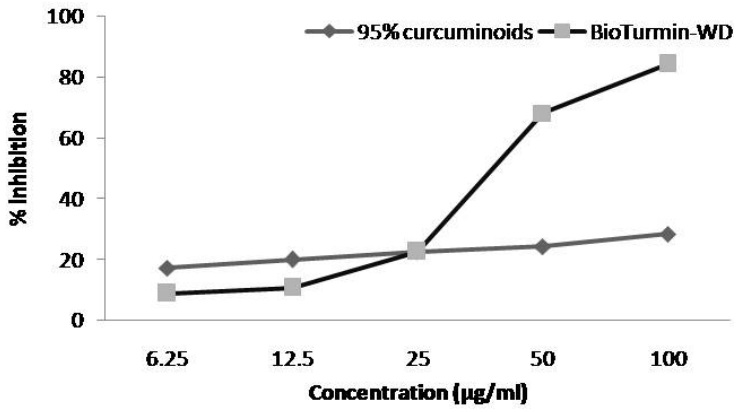

Figure 1. Percentage growth inhibition of curcuminoids by MTT assay

MTT: 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide.

3.2 Permeability Study

Permeable curcuminoids through Caco-2 cell monolayers were collected from basolateral part in different time intervals. Depending on the structure and molecular weight of the compounds, permeability of curcuminoids across the Caco-2 monolayer was varied. Percentage permeability and P_{app} values were calculated based on the ability of curcuminoids to cross the cell monolayers. Percentage permeability of BioTurmin-WD was found to be 0.294% diffused across the Caco-2 cell monolayer. Apical-to-basolateral direction P_{app} in Caco-2 cell monolayers of BioTurmin-WD and 95% pure curcuminoids has been represented in Table 1. Percentage permeability of BioTurmin-WD was found to be 3.46 times more compared to 95% pure curcuminoids diffused at 120 min. P_{app} of BioTurmin-WD was found to be 7.03 fold higher than that of curcuminoids.

Table 1. Permeability of curcuminoids across Caco-2 monolayers after 120 min

Sl. no.	Sample	dQ/dt (m)	A	C_0	Papp (cm/s)
1.	BioTurmin-WD	0.00002	1.13	3	5.89×10^{-6}
2.	95% curcuminoids	0.00003	1.13	10	2.65×10^{-6}

dQ/dt: Slope of the cumulative concentration of the compound in the receiving chamber over time (steady-state flux); A: Surface area of the porous membrane; C_0: Initial concentration applied in apical side; Papp: Apparent permeability coefficient.

4. Discussion

Scientific studies on curcumin, the active component of turmeric indicate that it is having relatively low bioavailability when administered orally (Sharma, Gescher, & Steward, 2005; Anand et al., 2007; Maheshwari et al., 2006). Curcumin undergo rapid metabolism to form conjugates such as curcumin glucuronides and curcumin sulfates in liver and intestinal wall. These metabolites may not have the same efficacy as that of curcumin. Most of the metabolites of curcumin are detected in plasma and serum rather than curcumin itself (Lao et al., 2006; Ireson et al., 2002; Ireson et al., 2001). Previous studies revealed that there are many approaches to enhance the bioavailability of curcumin and are as follows: 1) the use of piperine along with curcumin which interrupts the formation of curcumin conjugates; 2) micronization and the use of liposomal curcumin; 3) the use of nanotechnology; 4) the use of soy lecithin and microcrystalline cellulose in curcumin formulation and so on (Anand et al., 2007; Shoba et al., 1998). In the present study, a new preparation named as BioTurmin-WD containing water dispersible curcuminoids was assessed in order to foresee how the bioavailability could be improved. This new innovative preparation of curcumin has the following properties: 1) Easily dispersible in water; 2) It is both heat and light resistant; 3) It has no unpleasant odor or taste.

The bioavailability of BioTurmin-WD was evaluated in human Caco-2 cell monolayers, as an intestinal *in vitro* model system. The Caco-2 cell line exhibits a well differentiated brush border on the apical surface with the tight junctions and has proved to be the most popular *in-vitro* model (Meunier et al., 1995). Caco-2 cell model correlates with human intestine enterocytes and is used to predict oral absorption, and as a screening tool in drug discovery strategies for the prediction of intestinal drug permeability (Angelis & Turco, 2011). In order to study the bioavailability of water dispersible curcuminoids present in BioTurmin-WD, the test samples were applied to the apical side of a Caco-2 cell monolayer. The curcuminoids were quantified in the apical and basolateral sides after 2 h of incubation. BioTurmin-WD permeated the Caco-2 cell membranes was 0.2945% and the P_{app} value was found to be 5.89×10^{-6} cm/s.

The results obtained from the permeability studies, suggest that BioTurmin-WD has better bioavailability compared with 95% pure curcuminoids. Thus improved bioavailability is important for higher efficacy of the curcuminoids. Based on this when specified needs arise, developed formulation can be given to maintain the contentious uphold of the curcuminoids in blood. This water dispersible curcuminoid preparation, especially at a lower dosage, may be an ideal candidate to obtain benefits of curcumin. BioTurmin-WD disperses easily in aqueous system; as well as available for use in food and beverages.

5. Conclusion

The present study concluded that BioTurmin-WD shows 7.03-fold higher bioavailability than 95% pure curcuminoids through Caco-2 cell model. Hence, BioTurmin-WD may be safe to use as a dietary supplement to exert clinical benefits in humans at a lower dosage.

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