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AN IN-VITRO STUDY IN THE ENHANCEMENT OF CURCUMIN PERMEABILITY WITH PIPERIC ACID ACROSS CACO-2 CELLS

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ABSTRACT
Low molecular bioavailability of curcumin is a major issue regarding natural pharmacological agents. To overcome the barriers of bioavailability, many bio-enhancers have been used. Piperine is one among them. To make piperine more effective in this stream, hydrolyzed piperic acid was prepared and checked for permeability of curcumin.

KEYWORDS: curcumin, Piperine.

INTRODUCTION

Importance of natural products to be used as therapeutic agents as well as drugs is increasing nowadays. Many of them proved to be potential, but suffer from low bioavailability. The physical, anatomical and biological factors behind low bioavailability of most of the natural products have been established well. Physicochemical properties of the drugs, its interactions with foods, or chemicals, decomposition of the drug in the lumen, individual factors of the patient, hepatic first-pass effect, degradation in the gastrointestinal tract etc. are some of the important barriers for the drug bioavailability. Cell permeability of the drug is a key factor for effective drug delivery.[1] In order to overcome the barrier, many approaches have been attempted.

The drug penetration through the cell can be enhanced by the use of many chemicals such as DMSO, alcohols, glycols etc.[2] Most of these chemicals work by perturbation of intercellular lipid bilayers present in the epidermis.[3] Prodrugs are another method of increasing the lipophilicity and absorption of a polar drug.[4] Use of drug vehicles such as phospholipid vesicles- liposomes found to enhance the cell permeation.[5,6] While co-administrating with nutrient agents, some bioenhancers are found to increase the bioavailability. The important mechanisms behind the enhancement of bioavailability are found to be the reduction in hydrochloric acid secretion into the gastrointestinal tract, reduction in gastric emptying time, modifications in the epithelial cell membrane permeability of gastrointestinal tract, suppression of first pass metabolism and inhibition of drug metabolizing enzymes etc.[7-10] One important natural bioenhancer is piperine. It is an alkaloid, obtained from plants belonging to the *Piperaceae* family. The bioenhancer activity of piperine have mediated through different mechanisms such as DNA receptor binding, inhibition of drug metabolizing enzymes and production of glucuronic acid, inhibition of the proteins responsible for first-pass elimination of many drugs etc.[8,10,11] Many *in-vitro* permeation studies have proved the stated fact. In the study conducted, a much more bioenhancing - piperic acid, have been prepared and compared the permeability enhancement of piperic acid with piperine, co-administered with curcumin through human colon cell lines Caco-2 cell line.

Caco-2 cell line

The Caco-2 cell line is a continuous cell of heterogeneous human epithelial colorectal adeno carcinoma cells.[12] The cells derive from colon carcinoma- the epithelial cancer cells of large intestine and when cultured under specific conditions, they become differentiated and polarized. In this condition, they resemble the enterocytes lining the small intestine in their morphology and function, and can be used as a confluent monolayer - as an *in vitro* model of the human small intestinal mucosa to study the absorption of drugs.[13-15]

MATERIALS AND METHODS

The chemicals and solvents used were analytical grade. Piperic powder was prepared by the alkaline hydrolysis of piperine crystals in alcoholic medium, followed by neutralization. Caco-2 cells were sourced from NCCS, Pune.

The stock cells were cultured in DMEM supplemented with 20% inactivated Fetal Bovine Serum (FBS), penicillin (100IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5% CO_2 at 37^0C until confluent. The confluent

monolayer cultures were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks.

Cytotoxicity of different concentrations of test drugs was tested and a dose-response curve for each test substance was generated. The concentrations of test drug needed to inhibit cell growth by 50% (CTC50) values were calculated. The dose levels were fixed based on the cytotoxicity of test substances against CaCo2.

The test substance and Curcumin were weighed and suspended in alcohol to obtain final concentration of 5% curcumin with 5% of test substance. These dilutions were used for the permeability experiment.

Caco-2 cell monolayers were washed both in the apical and in the basolateral side with ringer solution. After washing, transport buffer was added to apical and basolateral parts and incubated at 37^0C for 30 min to attain equilibrium. Pre-warmed test substance 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 transferred to 37°C with 5% CO2 humidity incubator. At time intervals of 0 mins and 2hr, 100 µl samples were collected from basolateral side and fresh transport buffer of equal volume was replaced. The collected samples were properly labeled and stored at -20^0C for further analysis, using HPLC for the estimation of Curcumin content.

***Fig.1*. CaCo-2 permeability assay in Thincerts**

HPLC conditions

A reverse phase HPLC system consisted of waters 515 HPLC pump, a C_{18}, 250X4.6 mm, 5 µm column and waters 2489 detector was used for the analysis of curcumin content. Samples were prepared in methanol, injected 20 µl and eluted with an isocratic system of Acetonitrile: Water: Acetic acid (60: 39.5: 0.5), with a flow rate of 1.2 ml/min. The absorbances were made at 354nm. Permeability levels of each test substance were calculated from the Curcumin content in the samples, using the standard formula. The permeability potential of test substances were expressed as apparent permeability values in cm/sec and percentage permeability at 2nd hour.

RESULTS AND DISCUSSION

From the results, it is clear that curcumin have better permeability in presence of piperic acid with percentage permeability equivalent to 24.03 % with a P_{app} value **of 3.58x10^{-2}.** Whereas in presence of Piperine, Curcumin permeated with a lesser percentage permeability equivalent to 19.01% with respective $\boldsymbol{P_{app}}$ value 2.83X10^{-2} as given in Table 1.

Table 1: Permeability potential of Curcumin in presence of different test substances.

Sl. No	Test sample	CTC_{50} (µg/ml)	Apparent Permeability (P_{app}) in cm/sec.	% Percentage Permeability at 2nd hr.
1	**Piperine + Curcumin**	26.67±1.8	-2 2.83 X 10	19.01
2	**Piperic Acid + Curcumin**	42.33±3.8	-2 3.58 X 10	24.03

The results are plotted below

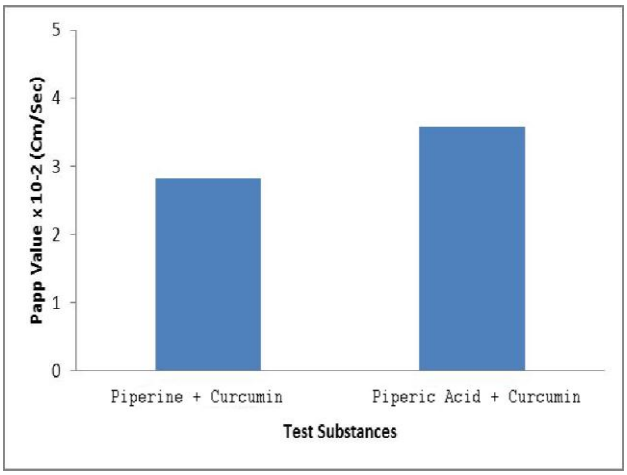

Fig.2. Apparent permeability of test susbtances in CaCo-2 cells at 2nd hour.

CONCLUSION

The current study to assess the permeability of test substances across CaCo-2 cells and reveals that, curcumin shows better permeability while co-administered with piperic acid, than piperine.

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