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Preparation, Characterization and Antiinflammatory Activity of Celecoxib-β-Cyclodextrin Inclusion Complexes

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The influence of β-cyclodextrin on the *in vitro* dissolution rate, *in vivo* absorption and oral bioavailability of a poorly water soluble antiinflammatory agent, celecoxib was studied. For this purpose, celecoxib and β-cyclodextrin complexes were prepared in 1:1 and 1:2 molar ratios by the physical mixture, kneading and freeze-drying methods. The complexes were preliminary confirmed using differential scanning calorimetry, fourier transform-infrared spectroscopy and scanning electron microscopy. The solubility studies revealed a linear relationship between the increase in celecoxib solubility and the increase in β-cyclodextrin concentration. The *in vitro* dissolution studies that were performed in phosphate buffer (pH 7.4) showed that celecoxib:β-cyclodextrin (1:2) solid complexes prepared by freeze-drying method had highest celecoxib release compared to the other solid complexes. Pharmacological studies were performed with this complex in a carrageenan induced rat hind paw oedema model. Regarding to the inhibition of edema (%) and swelling (%) results, celecoxib:β-cyclodextrin (1:2) binary mixture prepared by freeze-drying method showed significant improvement compared to pure drug.

Key Words: Celecoxib, β-Cyclodextrin, Solubility, Inclusion complexes, Antiinflammatory activity.

INTRODUCTION

Celecoxib (CEL) 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl] benzenesulphonamide, a cyclooxygenase-2 (COX-2) (Fig. 1) selective inhibitor is a non-steroid antiinflammatory drug (NSAID) approved for the treatment of rheumatic pain and osteoarthritis[1,2]. Most of NSADs inhibit both isoforms of cyclooxygenase (COX-1 and COX-2) and inhibit platelet aggregation. Because of CEL's specific COX-2 inhibition, it has less potential to cause gastropathy and risk of GI bleeding[3,4]. Celecoxib is a poor water soluble drug and it has a pKa 11.1 with low solubility

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(*ca.* 5 µg/mL). This fact contributes to high variability in absorption after oral administration and affects bioavailability. Thus, it is important to enhance the solubility and dissolution rate of CEL to improve its overall oral bioavailability[5]. There have been many studies recorded related to the enhancement of CEL solubility in water by using cyclodextrins[6-8].

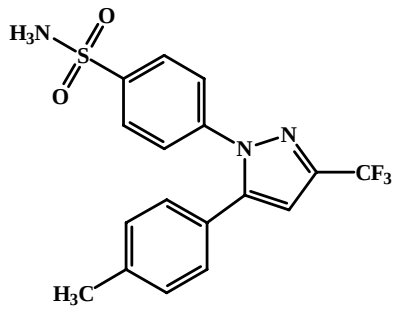

Fig. 1. Chemical structure of celecoxib (CEL)

Cyclodextrins are cyclic oligosaccharides composed of α-D-glucopyranose units joined through a 1-4 bond[9,10]. During the past decades, cyclodextrins and their derivatives such as β-cyclodextrin (βCyD) (Fig. 2), α-cyclodextrin and γ-cyclodextrin have aroused considerable interest in the drug formulation because of their potential to form complexes with many water insoluble drug molecules[11,12].

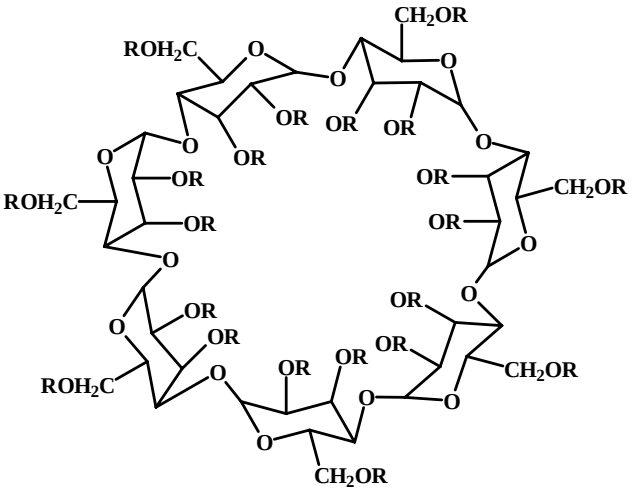

Fig. 2. Structural formula of β-cyclodextrin (βCyD)

They are capable of forming water-soluble inclusion complexes with poorly water-soluble drugs by taking up a whole drug molecule or a part of it, into the cavity of the cyclodextrin molecule[13-15]. Thus, they are commonly used in drug

formulations to enhance solubility, dissolution rate, stability and bioavailability by means of the formation of inclusion complexes[16-19].

The purpose of this study is to improve the solubility and *in vitro* dissolution rate, *in vivo* absorption and oral bioavailability of CEL. Drug and βCyD solid complexes were prepared in 1:1 and 1:2 molar ratios by the three different methods: physical mixture, kneading and freeze-drying. Complex formations were confirmed using differential scanning calorimetry, fourier transform-infrared spectroscopy and scanning electron microscopy. Dissolution studies were performed for binary complex systems and the overall results were compared with those of pure CEL and physical mixtures. Furthermore, based on the *in vitro* evaluation of complexes, the most suitable binary complex chosen for pharmacological studies and bulk drug was compared in the model of carrageenan induced hind paw edema in the rat.

EXPERIMENTAL

Celecoxib (CEL) was supplied by Pfizer (Turkey), β-cyclodextrin (βCyD) was purchased from Cerestar (USA). Other reagents were of analytical quality.

Phase solubility studies: Solubility study was carried out according to the method described by Higuchi and Connors[20]. To 10 mL distilled water containing various concentrations of βCyD (from 0 to 0.012 M) excess amount of CEL (30 mg) were added and sonicated in an ultrasonic bath for 20 min and shaken in water bath at 37 ± 0.5 ºC until equilibrium was reached after *ca.* 48 h. The suspensions were filtered through 0.45 µm Millipore membrane filter. CEL concentration was determined by HPLC[16]. The phase solubility was therefore obtained. The apparent 1:1 stability constant, Ks, was calculated from phase solubility diagrams using the equation[20]:

$$Ks = \frac{Slope}{So(1 - Slope)}$$

where So is CEL solubility in absence of βCyD (intercept).

Preparation of inclusion complexes in solid state: Based on the results of the phase solubility studies, solid systems were prepared with 1:1 and 1:2 molar ratios of CEL and βCyD, using kneading and freeze-drying methods. Physical mixtures were prepared as a reference for the purpose of comparison.

Physical binary mixture (PM): PMs were prepared by homogeneous blending (for 10 min) of previously sieved (60-160 µm) and weighed CEL and βCyD in a cubic mixer.

Kneading binary system (KNE): βCyD was wetted in a ceramic mortar with distilled water until a paste obtained. The required amount of CEL was then slowly added and the slurry was kneaded for about 1 h. Further, the product was dried at 37 ± 0.5 °C for 24 h and sieved through 125-500 µm sieve granulometric fraction.

Lyophilized binary system (LPh): CEL and βCyD were added to basic aqueous solution, according to 1:1 and 1:2 (CEL:βCyD) molar ratios. The solution stirring was maintained for 48 h. Furthermore, the resultant solution was filtered through

0.45 µm Millipore membrane filter, frozen and lyophilized in a freeze dryer (Lyovac GT-2, Leybold-Heraeus).

Characterization of samples

Thermal analysis by differential scanning calorimetry (DSC) was carried out using a Du Pont Instruments 910 apparatus. Samples (3 mg) of CEL, βCyD and binary systems were put into aluminium pans and scanned at the heating rate of 10 °C min^{-1} over the temperature range of 30-250 °C. FTIR spectrum of the CEL, βCyD, PMs, KNEs and LPhs were measured in potassium bromide disks using a Perkin-Elmer Model 1600 FTIR spectrometer (USA). SEM analysis of the CEL, βCyD and solid complexes were carried out using scanning electron microscope (Joel, JSM-5200, Japan) with an accelerating voltage of 20 kV and high vacuum operation. The samples were coated with gold-palladium mixture under an argon atmosphere at room temperature yielding a film thickness of 5 nm.

In this study, an HPLC method was developed to determine drug concentration released from CEL-βCyD solid complexes. For this purpose, method reported by Schonberger *et al.*[21] was applied after some modifications. The Shimadzu (Kyoto, Japan) LC 10A liquid chromatography system used in the analysis was consisted of a model LC 10 AT solvent delivery system, a Rheodyne injection system with a loop of 20 µL and a model CTO 10A column oven at 25 °C. The components were detected at 390 nm emission wavelength (252 nm excitation) by using model RF 10 AXL spectrofluori-metric detector. Separation was performed on analytical (250 mm × 4.6 mm) column with a guard column (3 mm × 4.6 mm) packed with octadecyl (particle size 5 µm), phenomenex. The mobile phase was acetonitrile-water (70:30) at a flow rate 1.2 mL/min. Modified method was applied without internal standard to obtain the calibration equation. Calibration curve was linear over a range of 0.2-10 µg/mL. For the determination of drug concentrations in samples, filtrated samples were diluted with ultra purified water and 20 µL portions were injected into the HPLC system. The drug concentrations were determined by peak areas using the standard curve established from a series of standards with known concentrations. Under these conditions, the polymer did not interfere with the drug peak.

***In vitro* dissolution studies:** The dissolution profiles of pure drug and binary systems were carried out in 900 mL phosphate buffer (pH 7.4), using Aymes DT-6E model dissolution apparatus, according to the USP rotating paddle method. Powdered samples containing 20 mg CEL were used. The paddle speed was set at 100 rpm and the temperature was maintained at 37 ± 0.5 °C. An aliquot of sample was withdrawn at specified time intervals, filtered through 0.45 µ nylon disc filter and analyzed by HPLC. Each test was carried out 3 times.

Pharmacological studies: The antiinflammatory activity tests were performed in order to determine the possible enhancement in pharmacological activity of CEL after incorporation into inclusion form. For this purpose, the animals were housed in plastic cages at constant temperature (22 ± 1 °C) and humidity (60 ± 1 %) under a 12 h light-dark cycle. They were given standard laboratory diet and tap water

ad libitum. The *in vivo* experimental protocol was approved by the Ethical Scientific Committee of Istanbul University, Cerrahpasa Medical Faculty and the experiments were carried out under veterinary supervision.

The antiinflammatory study was performed using carrageenan induced rat hind paw edema method on Wistar Albino rats (150-200 g)[22-24]. The animals were divided into 3 groups of 6 animals each. CEL:βCyD (1:2) LPh (equivalent to 20 mg/kg CEL) (Group 1) and pure drug (20 mg/kg) (Group 2) were administered orally as aqueous suspensions with 0.25 % sodium carboxymethyl cellulose. An aqueous solution of 0.25 % CMC was administered to control group (Group 3). After 0.5 h of oral irrigation of drugs, edema was induced by injecting 0.1 mL carrageenan (1 % w/v) into the plantar tissue of the hind-paw. The volume of the treated paw was measured with a plethysmometer at the scheduled time intervals for a period of 6 h and the swelling (%) and percentage inhibition of edema (%) were calculated with eqns. 1 and 2, respectively[25].

$$\text{Swelling (\%)} = \frac{Vt - Vo}{Vo} \times 100 \quad (1)$$

$$\text{Percentage inhibition of edema (\%)} = \frac{(Vt - Vo)_{control} - (Vt - Vo)_{treat}}{(Vt - Vo)_{control}} \times 100 \quad (2)$$

Vo = Average volume in the hind paws of rats before any treatment, Vt = Average volume after inflammatory agent injection.

RESULTS AND DISCUSSION

Phase solubility studies: The phase solubility diagram for the solid complex formation between CEL and βCyD is presented in Fig. 3. The graphical representation reveals a linear relationship ($R^2 > 0.99$) between the increase in CEL solubility and increase in βCyD concentration. The concentration of CEL in distilled water at pH 6.8, 25 °C is clearly affected by the presence of βCyD. According to the Higuchi and Connors classification, the diagrams obtained were AL type[20]. The apparent stability constant, Ks, obtained from the slope of the linear phase solubility diagram was found to be 1060.7 M-1, which indicates that, the CEL:βCyD complexes are adequately stable.

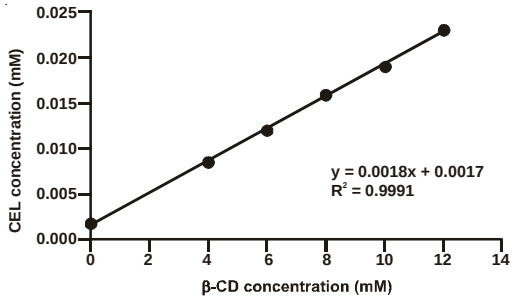

Fig. 3. Phase solubility diagram of CEL:βCyD in aqueous media at 37 ± 0.5 °C (n = 3)

Thermal analysis: Thermograms regarding to DSC analysis performed on CEL, βCyD and binary systems. The thermogram of CEL presents an endothermic peak at 164.2 ºC which represents the melting point of the drug and βCyD presents an endothermic peak at 119.4 ºC. Peaks related to CEL were observed to be reduced both in physical binary mixture and kneading binary mixture. More reduction of CEL peaks were also seen in CEL:βCyD (1:2) KNE formulations compared to 1:1. The peak reduction may be explained by small CEL content but its displacement is supposed to be caused by an interaction with the CD during heating in case of inclusion complexes. The thermograms of CEL:βCyD (1:1) lyophilized binary system (LPh) displayed nearly no peak corresponding to CEL melting whereas there was absolutely no peak in case of CEL:βCyD (1:2) LPh. This situation may be explained as the complete complex formation between CEL an βCyD when used in CEL:βCyD (1:2) LPh formulations.

Fourier transform-infrared spectroscopy (FTIR): FTIR spectra results of CEL, βCyD and binary systems were shown in Fig. 4. Characteristic bands of CEL were observed at 1596 cm^{-1} (-NH vibration), 1347 and 1134 cm^{-1} (SO_2 asymmetric and symmetric tension), 3099 cm^{-1} (C-H stretching band), βCyD spectrum presents a large band and peak in the region of 3900-2900 cm^{-1}, a shorter band between 1600 and 1700 cm^{-1} and a large band which displays distinct peaks, in the region of 1200-900 cm^{-1}. When the spectra of the PMs, KNEs and LPhs were analyzed, it can be easily seen that peaks related to -NH vibration at 1596 cm^{-1} were disappeared in all formulations tested. This could be due to the complex formation of CEL to

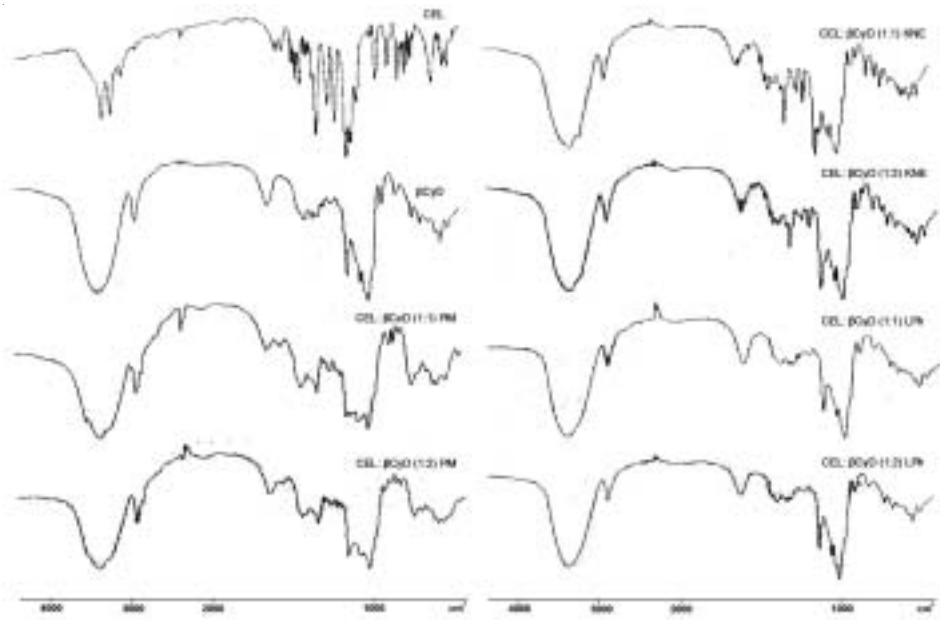

Fig. 4. FTIR spectras of CEL, βCyD and binary systems

βCyD from NH groups. In all complexes, C-O-R vibration band at 1638 cm^{-1} were seen due to βCyD and these bands disappeared in CEL. Strength of peaks in CEL:βCyD (1:2) LPh were lower than the one in 1:1. As expected, the lowest peak strengths were obtained in LPhs compared to KNEs and PMs. When peaks strength of CEL were analyzed, it could be seen that the highest peaks were observed which means that the lowering of CEL peaks strength show the complex formation between CEL and βCyD.

Evaluation of DSC and FTIR analysis suggest stronger interaction between drug and CD in the solid complexes rather than in a simple physical binary mixture, as a result of CEL:βCyD complexation.

Scanning electron microscopy (SEM): SEM is used to determine the microscopic properties of the CEL, βCyD and binary systems (Fig. 5). Therefore existence of the principal components in the complex and the possible enhancement effects of drying process on morphology of particles and solubility were analyzed. According to results obtained from SEM, pure CEL is characterized by the presence of a crystalline particle of regular size. Pure βCyD appears as crystalline particles without a definite shape. Physical binary mixtures, the characteristic CEL crystals, which were mixed with excipient particles or adhered to their surface, were clearly detectable, thus confirming the presence of crystalline drug. This fact was observed in physical mixtures between βCyD and other poorly water soluble drugs[26]. In case of KNE products, it was possible to differentiate CEL crystals gathered on the surface of βCyD particles that had lost their original shapes and crystal sizes were smaller. It was observed that LPh products appeared to show lesser crystalline structure

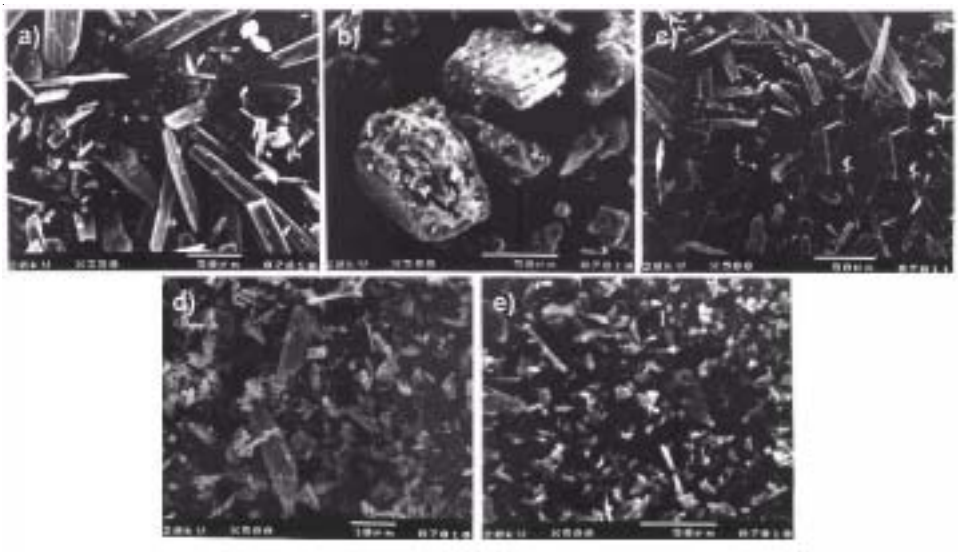

Fig. 5. SEM micrographs of CEL (a), βCyD (b), physical mixture (c), kneaded (d) and lyophilized (e) systems

with a soft and feathery appearance and crystals of the single components were still not detectable[27]. Lyophilized binary mixture seems to have acquired the same shape, presenting some sticks covering its surface, with a shape similar to that of the drug (Fig. 5).

Dissolution studies: The dissolution profiles of CEL and binary systems are given Fig. 6. CEL content in the solid complex formulations was assayed by a HPLC method which was developed to determine CEL content. The results of *in vitro* dissolution studies, CEL:βCyD (1:2) LPh showed the faster and highest CEL release (26.02 %) compared to the other formulations followed by CEL:βCyD (1:1) LPh (10.31 %), CEL:βCyD (1:2) KNE (6.11 %), CEL:βCyD (1:1) KNE (2.78 %), CEL:βCyD (1:2) PM (2.5 %) and CEL:βCyD (1:1) PM 2.03 %) at 15 min and 28.65, 13.42, 9.16, 5.53, 5.14, 3.19 % at 1 h, respectively. These results revealed that, released pure drug was 0.97 % at 15 min and 1.84 % at 1 h. Therefore solubility of CEL was enhanced by its complexation with βCyD as expected. The incorporation of CEL to βCyD in especially LPhs influenced its dissolution profile.

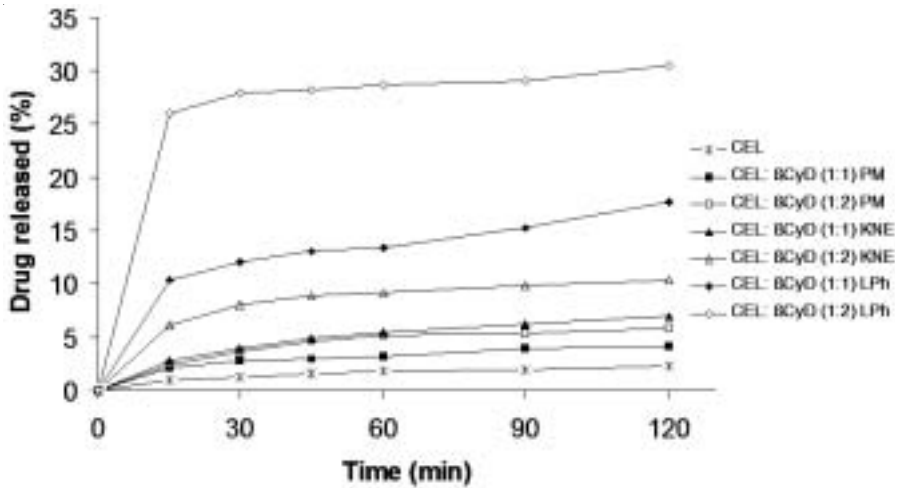

Fig. 6. *In vitro* dissolution profiles of CEL and binary systems (n = 3)

Per cent dissolved drug amount obtained with CEL:βCyD (1:2) LPh was found to give the best results especially when compared to PMs and pure drug, the amount dissolved was clearly high. This increase on CEL release is related to an improvement in its solubility caused by CEL:βCyD water-soluble inclusion due to preparation method and CEL:βCyD molar ratio.

When the two preparation methods were compared inclusion complex prepared with freeze-drying method, in both ratios, released the drug faster than the complex prepared with kneading method.

It was observed that the complexes with 1:1 ratio released less drug. However release rates of CEL were found very low both in physical binary mixtures and

which could be due to the incomplete complex formations. Similarly, CEL:βCyD (1:1) KNE formulation released drug very slowly as in CEL:βCyD (1:2) PM and not found suitable for use.

It is possible to reduce surface tension between soluble drug and dissolution medium. By using hydrophilic cyclodextrin and enhancement of drug's wettability and formation of easily soluble complex may improve the dissolution rate gained by physical binary mixture.

After preliminary studies, CEL:βCyD (1:2) LPh was found to be the most suitable one and chosen for *in vivo* experiments.

Pharmacological studies: Antiinflammatory activity tests were performed to compare the CEL's affectivity alone and inside inclusion complex. Results of these tests were shown in Table-1. The percentage inhibition gained by CEL:βCyD (1:2) LPh which was shown to have fastest and highest active substances solubility by *in vitro* solubility studies is more than 50 % in 1 h. It reaches the maximum value (66.85 %) at 2 h.

TABLE-1
RESULTS OF PHARMACOLOGICAL STUDIES

Time (h)	1 h	2 h	4 h	6 h
Control	18.10±3.09	32.66±2.51	40.45±7.65	48.38±13.04
Pure CEL	16.41±4.03(15.28)	29.96±7.28(20.23)	34.74±13.25(20.57)	40.12±11.58(16.69)
CEL:βCyD(1:2)LPh	8.48±1.16(53.08)	10.62±1.86(66.85)	16.92±5.09(55.50)	22.04±1.92(45.54)

Data as presented as means ± SD, n = 6, of swelling (%) calculated from eqn. 1, with percentage inhibition of edema (%) calculated from eqn. 2 given in parenthesis.

The swelling (%) were calculated 22.04, 40.12 and 48.38 % at 6 h for the CEL, CEL:βCyD (1:2) LPh and control group, respectively. When the swelling (%) and inhibition of edema (%) were analyzed, it can be easily seen that CEL:βCyD (1:2) LPh more effective than CEL for prevent of edema. However, both of therapy groups were achieved significant higher inhibition of the inflammation induced by carrageenan compared to control group ($p < 0.05$).

This fact may be attributed to the capability of forming water-soluble inclusion complexes with poorly water-soluble drugs by taking up the whole drug into its cavity in case of CEL. This mechanism improves the solubility and therefore CEL's poor water solubility changes and CEL dissolves more rapidly and in high amounts when complexed with βCyD. This result is in accordance with results obtained from solubility tests as expected.

Conclusion

According to these findings, it could be concluded that this is an important attempt for modulation of CEL release and further an improvement is expected on the bioavailability. Due to all the results obtained, it could be concluded that CEL solubility and dissolution characteristics might be modulated by using βCyD inclusion complexes. Additionally, *in vivo* test results demonstrated that anti-inflammatory activity of CEL:βCyD (1:2) LPh inclusion complex was found to be the most suitable.

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