Original Article

A comparison study of in-vivo pharmacokinetic parameters after oral cilnidipine nanocrystals administration in rats

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ABSTRACT

The purpose of the current research aimed to evaluate the in vivo pharmacokinetics parameters of the formulated cilnidipine nanocrystals (CLND NCs) after oral dosing in comparison to orally CLND dispersion. Cilnidipine is a Ca^{+2} channel blocker applied to control elevated blood pressure and its vascular prognosis. The solvent-anti-solvent method was utilized to prepare CLND NCs.The bioavailability parameters were measured in male Westar rats (n = 12), and each rat weighed between 200 ±20 grams. Rats were split up into two groups with an equal number in each (n=6). All groups fasted for twelve hours permitting water to be consumed before the trial. Blood samples (a half mL) were withdrawn from the retro-orbital venous plexus at (5, 15, 30, 45 minutes) and (1.0 2.0, 6.0, 8.0, 10.0, 12.0 hours) after receiving. Furthermore, the CLND plasma concentration of rats was calculated using HPLC. Findings showed that the relative bioavailability of CLD NCs was approximately 2.17 folds higher in comparison with the bulk CLND, and the maximum plasma concentration at (CPmax) was also increased. As well, the Tmax of the CLND NCs was less than pure drugs. The Conclusion achieved that overall results suggest that the developed CLND NCs was a proper mean for the promotion rate of dissolution and oral bioavailability of CLND.

Keywords: Cilnidipine, Nanocrystal, Surplus, PVPk90

Introduction

This was enabled by advancements in nanotechnology; a technique comprised of nanosuspension. The current strategy for improving compounds that are poorly soluble in water is called nanosizing. A minimizing in the particle size was guided to improve surface area exposure that takes place between the medium of dissolution and particles [1]. Lyophilization is a widely applicable technique in the pharmaceutical industry for intensifying stability [2-4]. Nanocrystals (NCs) were created by top–down, bottom–up, and mixed techniques. Bottom-up method has several benefits in comparison to the top-down process. They are minimal power, the method at the lower coast, and minimal equipment is necessary. They are convenient for labile substances as they run at minimal heat. As well, the particle sizes of NCs attained have a narrow distribution range [5]. Stabilizers added to the nanocrystal formulation should adhere to the drug particulate's nanocrystal surface and produce steric stabilization [6, 7]. The adaptation of a suitable approach for the formulation of nanocrystals relays on the physicochemical characteristics of the used polymer and the laden. However, the nanocrystal form has an advantage in their ability to consolidate the rate of dissolution and bioavailability of poorly soluble drugs [8, 9]. Cilnidipine is a Ca+2 channel blocker applied to control blood pressure elevation and its vascular prognosis. CLND is a Class II drug that possesses minimal water solubility and a low dissolution rate. It is a substantial lipophilic feature with a logarithm P value of 4.7 for this reason it holds proper gut permeation. Yet, the oral bioavailability of CLND was low [10].

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Materials and Methods

Materials

Cilnidipine powder (CLND), Soluplus, and PVPk90 were purchased from Hyperchem Chemical Company, Ltd (China). Acetonitrile (High-pressure Liquid Chromatography grade), CH3OH (High-pressure Liquid Chromatography grade), and ammonium acetate (High-pressure Liquid Chromatography grade, ≥99.0%) were acquired by (Alpha chemika, India). The rest compounds with solvent were of analytical reagent grade.

Formulation of CLND nanocrystals NCs

The CLND NCs formulas were formulated by the solvent-anti-solvent precipitation method. Different concentrations of CLND in water miscible solvent (methanol used in this study) were prepared, and then this solution was dropped into 50 ml of deionized water containing stabilizer (Soluplus and PVPk90) at a rate of 30 ml/hr as showed in **(Table 1)** [11]. Upon injection, precipitation of CLND NCs occurred immediately; every sample was run with an ultrasonic probe with a power input of 65 watts for 6 minutes (time on 6 seconds and 2 seconds off). The probe was soaked about 10-12 mm in the solution by which waves were moved downwards and reflected upwards, throughout ultra-sonication, the temperature was also conserved by utilizing a cool water bath and then subjected to freeze drying, to regain nanoparticles in dried shape from the nanosuspensions, water was carried away by lyophilization so that each formula was freeze-dried utilizing vacuum pressure of 2.5×10 Pascal about 48hour at a governed temperature of (- 45) °C. The produced powders were applied to further research [12, 13].

Table 1. Composition of CLD NCs

Ingredients	Amount
CLND	10 mg
Soluplus	20 mg
PVPk90	5 mg

In vivo pharmacokinetics

Animal experiments

The methods used to assess bioavailability parameters complied with the suggestions made by the National Committee for Research Ethics in Science and Technology (NENT, Norway) [14]. The bioavailability parameters were measured using male Wistar rats (n = 12), and each rat weighed between 200 ±20 grams. Rats were split up into two groups with an equal number in each (n=6). All groups fasted for twelve hours permitting water to be consumed before the trial. Ten mg of CLD powder had mixed in 40 mL of deionized water, and CLND NCs of 0.25 mg/ mL was dissolved, which were administrated orally by gavage tube to rat with a dosage of 2.0 mg/kg for both CLND dispersed powder and CLND NCs. Preceding oral administration all samples were shacked for 5 minutes to achieve complete dispersion. Blood samples (a half mL) were withdrawn from the retro-orbital venous plexus as shown in **Figure 1** at (5, 15, 30, 45 minutes) and (1.0 2.0, 6.0, 8.0, 10.0, 12.0 hours) following dispensation. Later on, blood samples were put into microcentrifuge tubes consisting of the anticoagulant (EDTA) and were instantly centrifuged at 4500 route per minute for 15 minutes in a cool centrifuge then samples were stored -25°C until HPLC analysis was performed [15]. The experiment was done in agreement with the ethical code of the World Medical Association (Declaration of Helsinki).

Bio-analytical technique

CLND of rat plasma sample was found utilizing the deproteinization approach. 10 microliter internal standard solution (200 ng/mL nimodipine solubilized in methanol) and addition of 500 µL acetonitrile to every 100 µL of melted plasma for protein to be precipitated. The components were stirred for 5 min and then centrifuged at 8,000 rpm for 10 min (Ohaus cooling centrifuge). The acquired supernatant was filtrated by a 0.22 µm filter membrane syringe and 20µL of filtered mixture was injected into the HPLC system for analysis. The mobile phase comprises 70% acetonitrile and 30% ammonium acetate solution (5 mM) and the flow rate was 0.3 mL /min. In case of detection of an unknown concentration of CLD in rat plasma which is collected during the procedure, the extraction of plasma samples is done by using the same method, and the plasma sample is spiking with 100 µl of mobile phase hold 5µg/ml internal standard (nimodipine). The unknown concentration of CLND could be calculated using the equation obtained from the spiked calibration curve. After measurements of CLND concentration in plasma with time, the analysis by using the non-compartmental technique to calculate pharmacokinetic parameters using PK-SOLVER. The drug's (CPmax) and time to CPmax (Tmax) and terminal half-life (t1/2) were calculated. AUC0-24 is the area under the plasma concentration-time curve from 0 to 24 hours [16].

Statistical analysis

Findings were demonstrated as mean values (±SD; n = 3). A statistically significant difference was taken into account when $p<0.05$. The pharmacokinetic parameters, Cmax, Tmax, AUC0-24, and AUC0-∞ were interpreted statistically utilizing a student t-test [17].

Results and Discussion

Spiked plasma samples calibration curve

The calibration curve was attained by using the proposed approach for the spiked plasma with a standard solution of known concentration of CLND. The findings of High-Pressure Liquid Chromatography analysis exhibited free overlapping of endogenous ingredients with the chromatograms of blank plasma,CLND and the internal standared nimodipine as displayed in **Figure 1**. Blank plasma chromatogram was detected

at 3.68 minutes as shown in **Figure 2**.The method was precise, specific, and sensitive for the calculation of CLND in the mobile phase standard solutions as shown in **Figure 3** and spiked plasma samples. The chromatogram of spiked plasma display complete separation of CLND, which displayed retention time (Rt) at 6.18 minutes from the internal standards (IS) nimodipine which displayed a peak at 2.18 minutes as demonstrated in **Figure 1**. The developed calibration curve demonstrated in **Figure 4** shows a straight relation between CLND concentration and the relative peak area of CLND to nimodipine, having a strong correlation factor (R2= 0.999) for five concentration points in the range of 3,5,8,12 and 20µg/ml. HPLC method was validated in the calculation of CLND amount in animal blood. All validation parameters were within acceptable criteria [18]. When HPLC-validated parameters were utilized, CLND was properly calculated. Retention persisted for 6.1 minutes. Five concentrations were applied to estimate the method's linearity and detect the lower limit of quantification, which was 5µg/ml [19].

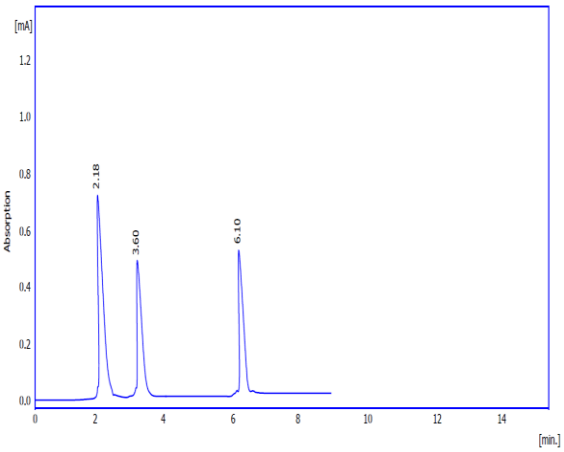

Figure 1. Spiked plasma samples Calibration curve with mobile phase including CLND and constant concentration of the internal standard (IS) (10 ng/ml).

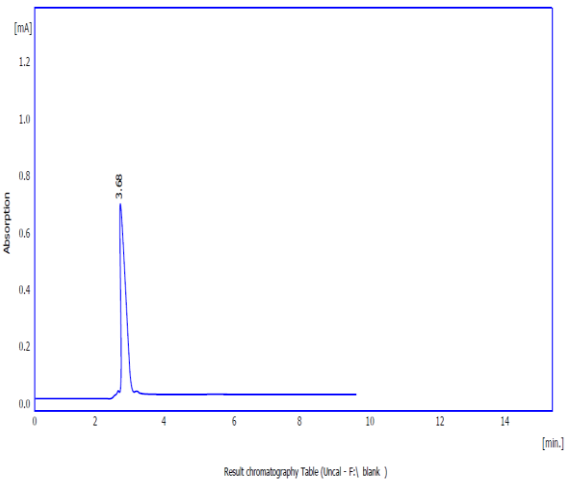

Figure 2. Blank plasma Chromatogram

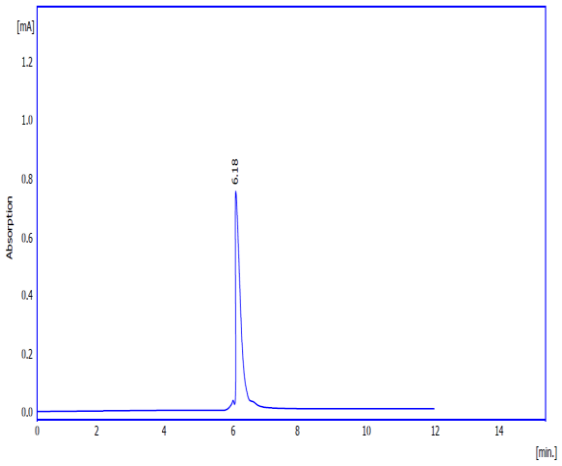

Figure 3. Chromatograms for CLND

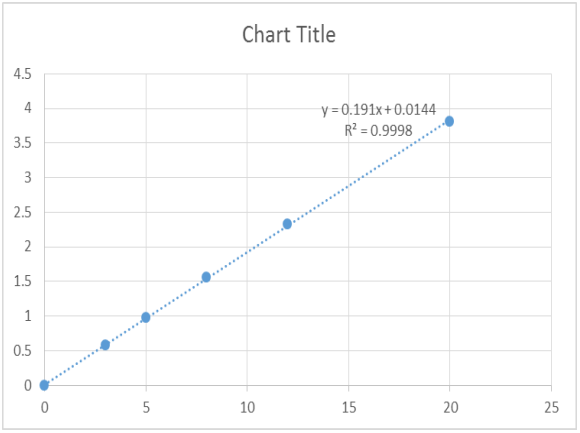

Figure 4. CLD Spiked Calibration Curve

In vivo pharmacokinetics

The in vivo pharmacokinetics of CLND and CLND NCs were estimated and compared in rats. A CLND plasma concentration-time profile was plotted and demonstrated in **Figure 5**, and the major pharmacokinetic parameters are epitomized in **Table 2**. As clear, the relative bioavailability of CLND NCs was approximately 2.17 folds higher in comparison to the bulk CLND, and the (CPmax) was also increased. As well, the Tmax of the CLND NCs was lower than the CLND pure drug. AUC0⟶∞ for CLND NCs was 601.159 while for CLND was 276.5. So, the recognized pharmacokinetic findings substantially evidenced that the oral bioavailability for CLND in rats has noticeably improved with CLND NCs formulation [20, 21]. With this research, the enhanced oral bioavailability of CLND NCs was supposedly due to several elements. First, since the reduction in particle size of CLND NCs and solubilization of surplus [22-24] the solubility and in vitro rate of dissolution considerably rose, which promotes oral bioavailability. Second, during CLND NCs passes the gut, and NCs become largely enhance the permeability of the membrane through raising contact surface area between NCs and gut barriers [25], or pass the gut biological barriers and get into the circulatory system [26-28].

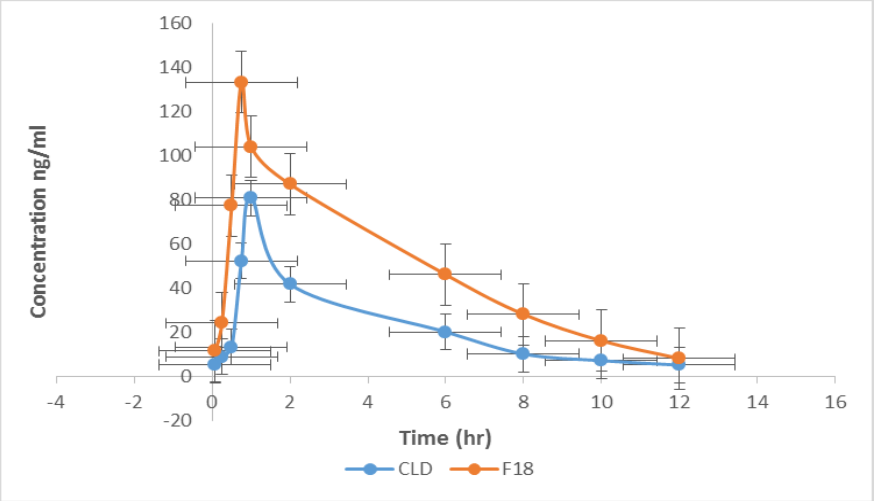

Figure 5. Rats' plasma concentration-time curves following oral receiving of bulk CLND and CLDNCs (mean ±SD).

Table 2. The pharmacokinetic Parameters

Parameter	CLND	CLND NCs
$C_{max(ng)}$	2	133
T_{max}	1	0.75
T ½ h	4	0.2
AUC0→12	247.6	575
AUC0→∞	276.5	601.159

Conclusion

The enhanced oral bioavailability of CLND NCs in rats was revealed by the subsequent in vivo pharmacokinetic investigation, the CLND NCs used in this research might be an appropriate approach for optimizing oral bioavailability and dissolution of CLND.

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Conflict of interest: None

Financial support: None

Ethics statement: The committee protocol in the College of Pharmacy/University of Baghdad approved this study, which complied with the ethics as reported in the guidelines written by the National Committee for Research Ethics in Science and Technology (NENT), Norway.

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