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Original Article

EFFECT OF METHYL-B-CYCLODEXTRIN COMPLEXATION ON THE HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF *KHELLIN*: EXPERIMENTAL STUDY

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ABSRACT

Objective: The present work tackled the development and evaluation of inclusion complex of *khellin* (KH) and methyl-β-cyclodextrin (MβCD). In addition, it tested its possible hypoglycemic and hypolipidemic effects.

Methods: Inclusion complexes of KH-MβCD in the presence of water-soluble polymer were prepared by freeze drying (FD), co-evaporation (EV) and kneading methods (KN). The selected ternary complex was characterized by Fourier transform infrared spectrophotometry (FTIR), x-ray diffractometry (XRD), differential scanning calorimetry (DSC) and scanning electron microscopy [1]. Assessment of the hypoglycemic effect of the selected ternary complex versus the standard drug metformin was studied. Two different doses of the ternary complex were administered orally to streptozotocin (STZ)-induced type 2diabetic rats. Their hypoglycemic and hypolipidemic effects were evaluated by measuring the fasting blood glucose level (BGL), total cholesterol (TC) and triglycerides levels (TG) along the study period.

Results: The FD complex showed the highest drug dissolution rate. All the performed characterization analysis confirmed the formation of a KH-MβCD inclusion complex. The *in vivo* study declared that both doses showed a marked hypoglycemic and hypolipidemic effects compared to metformin.

Conclusion: In conclusion, this study points for the first time that the complexation of KH with MβCD could notably improve the dissolution rate and hence the bioavailability of KH. Moreover, this study demonstrated that this compound has a hypoglycemic and hypolipidemic effect. Thus, it can be a promising natural supportive treatment in type 2 diabetes mellitus (T2DM).

Keywords: Khellin, Cyclodextrin complexation, Methyl-beta-cyclodextrin Hypoglycemic effect, Hypolipidemic effect

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INTRODUCTION

Diabetes mellitus is a serious health problem [2]. Diabetes is considered as a disease symptomized by hyperglycaemia caused by a deficiency in insulin production, its activity or both [3-5]. It is a common and widespread disease in developed and developing countries [6]. About 382 million people across the globe are reported suffering from diabetes mellitus [7]. This number is estimated to reach 592 million by the year 2035 [8]. As predicted by the World Health Organization (WHO); this disease will become the 7th among the leading causes of deaths worldwide [6, 9].

The most abundant form of diabetes is T2DM which accounts for almost 90% of all reported diabetes cases [10]. T2DM patients usually face a number of challenges represented in the direct and indirect effects of their diabetic conditions [11]. These complications varied from mild to severe ones, e. g., diabetic retinopathy, neuropathy, nephropathy and cardiovascular diseases leading to high rates of morbidity and mortality among diabetic patients [12, 13].

For management of T2DM, oral dosage forms are typically the first medications used, rather than the insulin injections, having a wide range of efficacy, safety, in addition of the overwhelming preference of the patients [14]. Over the last few years, oral antidiabetic therapy for T2DM has transformed from sulfonylureas as a single option to different classes of drugs including glinides, biguanides, thiazolidinedione's and a-glucosidase inhibitors [15]. Most of the commonly available drugs are usually accompanied by many side effects as edema, weight gain, anemia, heart failure, and gastrointestinal intolerance [16]. Therefore, several approaches are presently available to ameliorate diabetes and its complications reducing hyperglycemia, however, there is a demanding need to find out novel classes of active compounds [17-20]. The WHO has recommended the evaluation of active ingredients of plant origin to produce safe, modern drugs which have led to an urgent need for exploring new natural antidiabetic medications to minimize or even eliminate the possible side effects [21, 22].

KH is a chemical compound obtained from the plant *Ammi Visnaga* which has traditionally been used in Egypt. It is mainly used as a remedy for kidney stones by slowing the buildup of calcium oxalate and by acting as a diuretic [23, 24]. Advanced studies identified derivatives of KH to treat different types of tumors, epileptic seizures and inflammatory diseases [25-27]. Moreover, a previous study performed on the aqueous extract of *Ammi Visnaga* proved its significant antidiabetic effect in both normal and diabetic rats [28]. Unfortunately, the limited bioavailability of KH due to its poor water solubility and retarded dissolution rate lead to failure in the achievement of target drug levels and the maintenance of effective therapeutic concentration.

Cyclodextrins (CDs) has been effectively explored as complexing agents to promote the bioavailability through the enhancement of both the solubility and dissolution of different active ingredients [29, 30]. CDs are cyclic oligomers formed primarily of six to eight d-glucose monomers attached together by α-1, 4-glucose bonds. Such oligomers are characterized by a central hydrophobic core surrounded by a hydrophilic outer surface permitting the encapsulation of a wide variety of drug molecules forming host-guest complexes [31, 32]. This complexation allows the reduction of the dose of the administered drug minimizing the expected side effects [33, 34]. Furthermore, significant improvement in the solubilizing power of CDs, as well as its complexation efficiency in aqueous solutions, was previously reported by the addition of water-soluble polymers [35, 36].

KH is a drug of multiple pharmacological effects representing a very good applicant for wide range of pharmaceutical applications [37].

However, its poor aqueous solubility acts as a barrier preventing further investigations. Therefore, the current study was designed to explore the feasibility of enhancing the solubility and dissolution profile of KH aiming to produce a better candidate for more pharmaceutical formulations. Moreover, the study was concerned with investigating the effectiveness of KH as a hypoglycemic and hypolipidemic agent which has not yet been studied in any available research work. Inclusion complexes of KH with CD in combination with different water soluble polymers were prepared. The effect of the method of complexation, as well as that of water soluble polymers on solubility and complexation efficiency, was evaluated. Furthermore, the hypoglycemic and hypolipidemic effects of the drug-CD complex were evaluated by measuring the BGL, TC, and TG levels of STZ induced diabetic rats.

MATERIALS AND METHODS

Animals

Wistar male rats weighing 150–200 g were used for the study. They were housed under standard environmental conditions where the temperature and relative humidity were kept at 26±2 ^{0}C and 45–55 % respectively. Animals were allowed for standard laboratory diet and water *ad libitum*. The experimental protocol of the study was reviewed and approved by the Animal Ethics Committee of the National Research Centre.

Chemicals agents

KH was kindly provided by Memphis Pharmaceutical Company, Cairo, Egypt. MβCD was obtained as a gift from Roquette, France. Hydroxypropyl methylcellulose (HPMC) and Polyvinylpyrrolidone (PVP) were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals were of analytical grade, obtained from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Phase solubility studies [38]

Phase solubility of KH in MβCD was carried out by adding an excess amount of KH (100 mg) to 5 ml of aqueous solutions containing increasing concentrations of MβCD (0, 5, 10,15, 20, 30, 40 and 50 mmol) in tightly sealed glass vials. The vials were stirred using a horizontal shaker water bath at 100 rpm maintained at 37±0.5 °C for 72 h (Memmert GmbH, Germany). After the equilibrium had been reached, the suspensions were centrifuged at 7000 rpm for 30 min (Union 32R, Hanil Science Industrial Co., Korea). Thereafter, the dispersions were filtered through a 0.45 µm membrane filter (Millipore®, Spain) to obtain a clear solution. The filtrates were diluted with 60% (v/v) ethanol, and for the determination of the amount of solubilized KH, the absorbance was measured at 278 nm using spectrophotometer (Shimadzu UV spectrophotometer, 2401/PC, Japan).

The apparent stability constant (K_c) of the KH-MβCD complexes were calculated from the slope of the phase-solubility diagrams according to the following equation [38]:

$$K_c = \text{Slope}/S_0\ (1-\text{Slop})$$

Where S_0 is the intrinsic solubility of KH in water (solubility of KH in absence of CD).

Effect of water soluble polymers on solubility of KH [1]

To study the effect of water-soluble polymers; PVP and HPMC on the MβCD complexation of KH, the solubility of the drug were determined as previously mentioned. Briefly, 100 mg of KH was added to 5 ml of aqueous solutions containing increasing concentrations of MβCD (0, 5, 10, 15, 20, 30, 40 and 50 mmol) in tightly sealed glass vials containing PVP or HPMC in two different concentrations; 0.25 and 1 % (w/v). The suspensions formed were heated in an autoclave to 120 °C for 20 min, and then allowed to equilibrate using a horizontal shaker water bath at 100 rpm maintained at 37±0.5 °C for 72 h. After equilibration had been attained, an aliquot of the suspension was filtered through a 0.45 µm membrane filter, diluted with 60% (v/v) ethanol and spectro-photometrically measured at 278 nm.

Preparation of KH-MβCD ternary inclusion complexes

The solid complexes of KH and MβCD were prepared at 1:1 molar ratio containing 0.25% HPMC (w/v) using following methods:

Freez-drying method [39]

The complex was prepared by dissolving the MβCD and HPMC (0.25%w/v) in 60 % (v/v) ethanol. KH has dispersed in the ethanolic solution in suitable proportions KH/MβCD molar ratios 1:1. The dispersion was stirred at 25^{0}C for 24 h. The solution was prefrozen in a deep-freezer (Sanyo Ultra-Low-Temperature Freezer MDF-192, Osaka, Japan)-80 ^{0}C overnight and lyophilized in a freeze dryer (Labconco Corp., Kansas City) for 48 h. The complex was stored in sealed containers until further use.

Kneading method

MβCD, KH (MβCD/KH ratio 1:1) and HPMC (0.25%w/v) were ground and mixed thoroughly for 15 min in a glass mortar. The produced blend was kneaded for 30 min with 60 % (v/v) ethanol. The paste formed was dried under vacuum for 24 h. Dried powder was sieved through sieve no. 60 and stored in sealed containers until further use.

Co-evaporation method

MβCD, KH (molar ratio 1:1) and HPMC (0.25%w/v) were dissolved in 60 % (v/v) ethanol and stirred using magnetic stirrer at 45^{0}C for 6 h. The paste was dried overnight in a vacuum desiccator. The dried powder is ground then sieved through sieve no. 60 and stored in sealed containers until further use.

A physical mixture of KH and MβCD (1:1 molar ratio) with 0.25% HPMC (w/v) was prepared by thoroughly mixing the components in a glass mortar for 15 min.

Molecular modeling [40]

A molecular Docking algorithm was performed in order to study and verify the inclusion performance of guest (KH) into the host (MβCD) using a CHARMm-based MD docking algoritm [41]. It was performed using the Discovery studio 2.5. Cartesian coordinates of the host (MβCD) and the guest were extracted and built from the ChemBio Office Ultra 12.0. The host (MβCD) and the guest were optimized by semiempirical method (AM1) using Chem3D to eliminate bond length and bond angle biases and saved to be used in docking and binding energy calculations. To mimic the inclusion mode, MβCD and KH was separately defined as receptor and ligand, and then the binding site of MβCD was specified by a site sphere at the centroid of the narrow rim with a radius of 6 angstroms. Simultaneously, the CHARMm force field was applied to both MβCD and KH. The docking poses were achieved using CDOCKER protocol by placement of the rigid conformation of KH over combinations of rotational and translational motions within the grid box in MβCD.

In vitro dissolution studies

Dissolution studies were carried out following the USP XXII paddle method using a dissolution tester (Hanson SR8plus, USA). 20 mg KH or its equivalent of the complex or physical mixture in transparent hard gelatin capsule number (0) was used. The dissolution of capsules was tested in 500 ml of phosphate buffer pH 7.4 at 100 rpm maintained at 37±0.5 °C. At specified time intervals, an aliquot of 5 ml was withdrawn and replaced immediately with an equal volume of dissolution medium to maintain total volume constant. The withdrawn samples were filtered through 0.45 µm millipore filter and analyzed for drug content spectrophotometrically at 278 nm after appropriate dilution. Dissolution profiles were plotted and cumulative amount of drug dissolved as well as dissolution efficiency was calculated by the following equation [42]:

$$\text{D. E.} = \frac{\int_{t1}^{t2} y\ dt}{y_{100}(t2 - t1)} x100\%$$

Where y is the percentage of dissolved KH.

Characterization of inclusion complex in solid state

X-ray diffractometry (XRD)

X-ray powder diffraction patterns were recorded on a Diano X-ray diffractometer equipped with Co Kα (USA). The tube was operated at 45 kV, 9 mA [43].

Differential scanning calorimetry (DSC)

DSC analysis was performed using Shimadzu DSC-60 (Kyoto, Japan). 4 mg samples were placed in sealed aluminum pans, before heating under nitrogen flow (40 ml/min) heated in the range of 30-300 °C at a heating rate of 10 °C/min. An empty aluminum pan was used as a reference.

Scanning electron microscopy [1]

The shape and surface morphology of studied samples were evaluated using scanning electron microscopy (JXA-840 A, JEOL, Tokyo, Japan) operated at 25 kV. All samples were made electrically conductive by coating with a thin layer of gold with sputter coater (Edwards-S1150A) before being examined using various magnifications.

Fourier-transform infrared spectroscopy (FT-IR)

FTIR spectra were recorded on Fourier transform infrared spectrometer (Perkin Elmer, MA, USA). The samples were mixed with potassium bromide (KBr). The KBr discs were prepared by compressing the powders at a pressure of 10 tons for 5 min in a hydraulic press. The FTIR measurements were performed in the scanning range of 4000–400 cm^{-1} at ambient temperature with a resolution of 4 cm^{-1}.

Assessment of hypoglycemic and hypolipidemic effects of selected KH-MβCD ternary solid complex

Streptozotocin (STZ) induced hyperglycemia

Diabetes was induced in overnight fasted rats but allowed free access to water. The rats were administered intraperitoneally with a multiply low dose of STZ (50 mg/kg b.w. dissolved in 0.01M citrate buffer pH 4.5) [44, 45]. Blood samples collection were done the third day following diabetes induction from the retro-orbital plexus in capillary tubes (Micro Hemocrit capillary, Mucaps). Fasting blood glucose levels were measured and the rats with blood glucose levels ranging from 200-250 mg/dl were considered to be diabetic and were used in the study.

Blood samples were collected by standard method for estimation of serum triglycerides and cholesterol by using commercially available diagnostic kits.

Experimental design

The animals were divided to 5 groups of 6 rats in each group.

Group I: received saline and served as normal control. Group II: received saline and served a negative diabetic control. Group III: diabetic rats received antidiabetic drug metformin served as positive control. Group IV and Group V: diabetic rats received tested complex at doses 100 mg/kg and 200 mg/kg respectively.

Drugs were orally administrated to rats daily for 14 d which were allowed free access to food and water *ad libitum*. On the 15th day, rats were sacrificed, and blood samples were collected from the retro-orbital plexus used for the measurement of glucose, triglycerides, and total cholesterol. Glucose, triglycerides, and cholesterol were estimated by enzymatic methods using diagnostic kit [46].

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by the least significance test (LSD). This statistical analysis was computed using SPSS 16.0®software.

RESULTS AND DISCUSSION

Phase-solubility studies

The results indicated that the aqueous solubility of KH increased linearly with increasing the concentration of MβCD (fig. 1). The solubility curve has shown a slope of 0.0023 having a correlation coefficient value of 0.9912 (r^2>0.990). According to the definition set by Higuchi and Connors [38], the phase-solubility diagram could be classified as A_L type having a slope<1 thus resulting in the observed enhancement of solubility due to the development of 1:1 complex. The apparent stability constant (Kc) of KH–MβCD complex (1:1) was 13.18 M^{-1} indicating the stability of the inclusion complex formed between KH and MβCD.

Effect of water soluble polymers on solubility of KH

The effect of PVP and HPMC as two water-soluble polymers on the solubility of KH in KH-MβCD complexation was investigated. The solubility profile of KH in the presence of 0.25 and 1% w/w PVP or HPMC are illustrated in fig. 1. As depicted from the fig., the addition of PVP or HPMC with two different concentrations to the cyclodextrin solution has not altered the type of phase solubility diagram observed for the binary system. Kc values calculated were found to be 39.71, 13.18, 78.94 and 19.80 $M^{-1}$for 0.25% PVP, 1%PVP, 0.25%HPMC and 1% HPMC respectively.

From these results, it can be seen that the solubility of KH in the presence of cyclodextrin was increased from 13.18 M^{-1} to 78.94 M^{-1} upon the addition of 0.25% HPMC. These obtained results are in accordance with previously reported studies [1, 35] proving that the incorporation of water-soluble polymers in small amounts has enhanced the solubilization and complexation power of cyclodextrins. The noticed increase in Kc upon the addition of HPMC shows that the polymer is able to interact differently with the drug-cyclodextrin binary complex through hydrogen bridges or van der Waals forces playing an important role in complex formation [47].

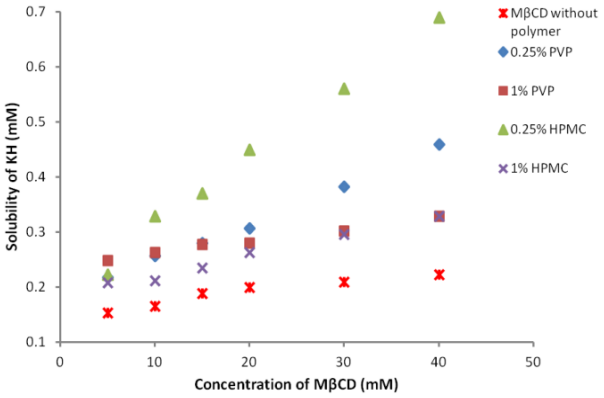


Fig. 1: Phase solubility diagram of KH with MβCD and water soluble polymers

Preparation of KH-MβCD inclusion complexes

The solubility studies proved the formation of a 1:1 inclusion complex between KH and MβCD molecules. Moreover, the addition of 0.25% HPMC resulted in a significant increase in the aqueous solubility of KH. Therefore, the theoretical molar ratio of 1:1 inclusion complex in the presence of 0.25% HPMC was chosen to prepare the solid complexes using different methods. Three complexes were prepared; FD by freeze drying method, KN by kneading method and CV using co-evaporation method. The three complexes were subjected to further investigations compared to the physical mixture (PM).

Molecular modeling

The corresponding CDOCKER interaction energy (Kcal/mole) and hydrogen bond (H-bond) formation of KH were considered in our study to prioritize their virtual optimum arrangement inside the hydrophobic cavity of MβCD resulting from docking were shown in fig. 2. Results revealed that these novel compounds have the ability of Docking study revealed that this guest (KH) has good docking score in addition to its ability of formation four intermolecular hydrogen bonds acceptors (O H· · ·O) detected in KH/MβCD inclusion complex between the hydroxyl group of CD and the oxygen atom of KH. The hydrogen bonds length were ranging from 2, 13-2.44 A °) which reflect the strength of this interaction leading to the stability of KH/MβCD complex (table 1).

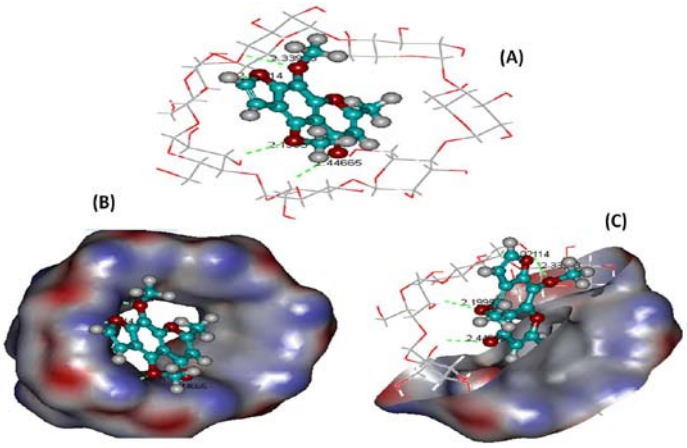
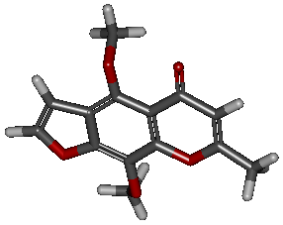


Fig. 2: Outline of the molecular docking. (A) Three-dimensional structure of the guest (KH) and the host (MβCD); (B) The guest is docked into the binding cavity of the receptor; (C) Cutaway view of CD hydrophobic cavity showing KH inserted in the cavity of MβCD

Table 1: CDOCKER interaction energy scores, hydrogen bonds and energy for guest (KH) compound docked into host (MβCD)

Guest (KH)	Absolute Energy (Kcal/mole)	CDOCKER interaction energy (Kcal/mole)	hydrogen bonds
	106.86	-22.90	4 H-bonding acceptors with OH of MβCD (2.13-2.44 A °)

***In vitro* dissolution studies**

The dissolution profiles of KH-MβCD ternary complexes containing 0.25% w/w HPMC prepared by three different methods compared to KH powder and the physical mixture of KH and MβCD at the same ratio are shown in fig. 3. As observed from the profiles, the dissolution rate of KH in FD and CV complexes was clearly higher than that of the drug alone, PM and KN complex. Pure KH exhibited a very low dissolution rate where less than 20% and 25% of KH were dissolved after 30 and 60 min. On the other hand, some dissolution improvement was observed in the case of PM showing the dissolution of more than 20% and 30 % of KH after 30 and 60 min. The enhanced dissolution profile of the PM is likely due to the presence of MβCD where the surfactant properties of CDs, increased the wettability and thus reduced the interfacial tension between drug and dissolution medium, resulting in a subsequent increase in the drug dissolution rate [48, 49].

In contrast, the prepared complexes exhibited much faster dissolution compared to pure KH. These results are in accordance with the data obtained from the phase solubility study proving the enhanced solubility of KH in the presence of MβCD. Among the prepared complexes, FD showed the highest dissolution profile with an accumulative dissolution more that 85% and 90 % after 30 and 60 min. The observed increase of KH dissolution profile obtained in case of FD complex was probably due to amorphization of the drug by applying the freeze-drying method resulting in better wettability and consequently increases the drug solubility which likely contributed to the enhanced dissolution of the complex [50, 51]. Also, the increase in dissolution efficiency can be attributed to the reduction of crystalline nature of KH [52]

A comparison between the complexes prepared by various methods was made by determination of the dissolution efficiency (D. E.) [53]. Considering the D. E. values (table 2), the dissolution rate of KH increases in the order: KH<PM<KN<CV<FD complexes suggesting that dissolution rate was influenced by the method used for the preparation of complexes. As manifested from the results, D. E.30 and D. E. 60 were the highest for the FD complex.

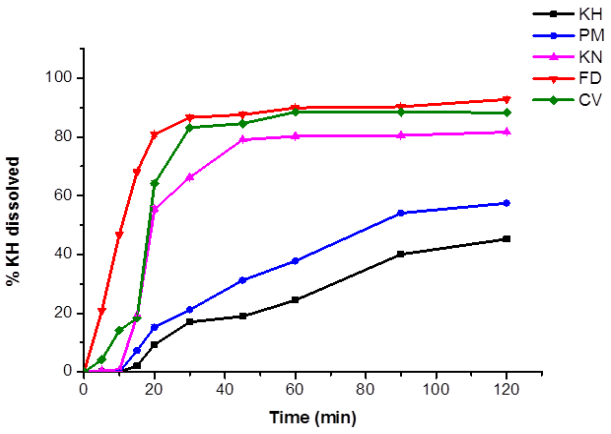


Fig. 3: Dissolution profiles of KH, physical mixture (PM), Kneading (KN), co-evaporation (CV) and freeze drying (FD). Results are expressed as means±SD (n=3)

Table 2: Dissolution efficiencies of KH and KH-MβCD complexes

Dissolution efficiency (D. E.)±SD	KH	PM	KN	CV	FD
D. E.% $_{(0\text{-}30)\pm SD}$	6.47±0.03	10.43±0.55	34.36±0.91	42.22±0.34	66.24±0.42
D. E.% $_{(0\text{-}60)\pm SD}$	13.45±0.30	21.07±0.02	55.85±0.94	63.94±0.11	77.23±0.23

Results are expressed as means±SD (n=3).

Characterization of inclusion complex in solid state

Differential scanning calorimetry (DSC)

The thermal behavior of KH compared to its freeze-dried complex as well as its physical mixture together with MβCD and HPMC were illustrated in fig. 4. The DSC results demonstrated a well-defined endothermic peak for KH at 153.5^0 C corresponding to the melting point showing a typical behavior of an anhydrous crystalline drug (fig. 4a). The DSC curve of MβCD exhibited a broad endotherm in the range of 50C to 150^{0}C, due water loss, proving its amorphous hydrated state[54] (fig. 4b). A broad endothermic peak for HPMC due to the dehydration process was also observed over a temperature range of 30-110^{0}C (fig. 4c). From the DSC curve of the physical mixture (fig. 4d), it is possible to observe two endothermic peaks nearly identical to that of pure KH and MβCD. The reduction of the endothermic peak of KH suggests that the heat produced during DSC scan resulted in an interaction between KH and MβCD leading to the loss of some of the crystallinity of KH and the development of a new solid phase, which melts at a lower temperature compared to KH. The thermogram of the freeze-dried complex illustrates the absence of the characteristic endothermic melting peaks of both KH and MβCD indicating the amorphous character of the complex and proving the inclusion complexation of the KH inside the MβCD cavity[55] (fig. 4e).

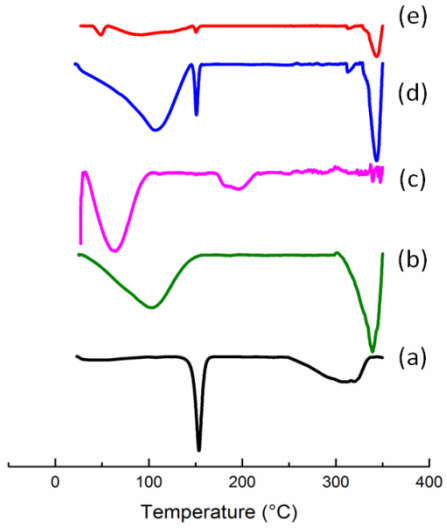


Fig. 4: DSC thermograms of KH(a), MβCD(b), HPMC(c), physical mixture (d) and freeze drying (e)

X-ray powder diffractometry (XRPD)

The XRPD patterns for KH, MβCD, HPMC and the physical mixture, as well as the freeze-dried complex, are presented in fig. 5. The diffraction pattern of KH showed two sharp characteristic peaks of higher intensity at 2θ=9.33 ° and 24.85 °, indicating the crystalline behavior of the drug (fig. 5a). The amorphous nature of both MβCD and HPMC is evident from the absence of sharp, distinct peaks characteristic to crystalline compounds (fig. 5 b and c). The physical mixture profile is characterized by the presence of combined overlapping peaks of KH and MβCD, however, with reduced intensities, showing that KH maintained its initial crystallinity (fig. 5 d). These changes may be attributed to a reduction in particle size and dilution of the pure crystalline components during the physical mixture preparation [56]. On the contrary, the characteristic peaks of KH can no longer be distinguished in the complex diffraction pattern (fig. 5e), thus suggesting the inclusion of KH into the CD core, indicating the production of an amorphous inclusion complex. Furthermore, a reduced number of signals were noticed in the complexes which is a good indication on the greater amorphousness of the inclusion compounds compared to the uncomplexed molecules [57].

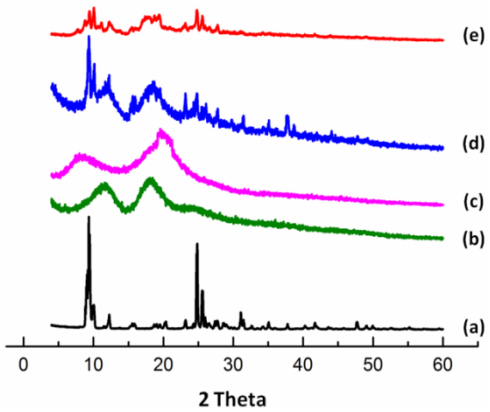


Fig. 5: X-ray diffraction patterns of KH(a), MβCD(b), HPMC(c), physical mixture (d) and freeze drying (e)

Scanning electron microscopy [1]

SEM photographs of KH, MβCD, physical mixture and freeze-dried complex are shown in fig. 6. The micrographs confirmed the crystalline nature of KH which appeared as rectangular plate-shaped crystals while MβCD is presented as spherical particles with cavity structure (fig. 6 a and b). The physical mixture of KH-MβCD revealed the characteristic crystals of KH, which were blended with those of MβCD molecules or attached to their surface (fig. 6c). Irregular shaped bulky particles were noticed in the case of the FD complex with the disappearance of the characteristic morphology of both KH and MβCD indicating an apparent interaction between KH and MβCD resulting in the formation of a new solid inclusion complex (fig. 6d).

Fourier transform infrared (FTIR)

FTIR spectra for the (a) KH, (b) MβCD,(c) physical mixture and (d) FD complex of KH-MβCD (fig. 7). The spectrum for the investigated complex appeared approximately the same as MβCD declaring the production of inclusion complex; same observation was recorded by Li *et al.* [58]. KH crystals show three absorption bands at 2927.41 cm^{-1} corresponding to C=H bond, 1646.91 cm^{-1} corresponding to carbonyl stretching vibration (C=O) and 1061.62 cm^{-1} corresponding to C-O bond. Absorption band of KH appearing at 1061.62 cm^{-1} became broader and shifted to a higher wave number in case of the complex which is a good indication of the presence of host-guest interaction. However, the two other bands of KH became sharper. As well, a broad hydroxyl band of MβCD at 3411.46 cm–1 was noticed to be narrowed in the spectrum of FD complex pointing to inclusion complex formation.

Assessment of hypoglycemic and hypolipidemic effects of selected KH-MβCD ternary solid complex

Hypoglycemic effect on blood glucose level of STZ-induced types 2 diabetic rats

The potential hypoglycemic effect of the selected KH-MβCD freeze-dried complex was evaluated in STZ-induced type 2 diabetic rats. To find out the optimum dose, two different doses of KH-MβCD freeze-dried complex (100 and 200 mg/kg b.w.) were examined compared to metformin as a standard drug (500 mg/kg b.w.). The effect of both doses of the complex and metformin up to 14 d in mild diabetic rats was studied (table 3). Oral administration of KH-MβCD freeze-dried complex at both doses resulted in a significant reduction in blood glucose level (BGL) when compared to diabetic control rats on the 7th day ($p<0.05$). However, no significant difference was observed upon administration of metformin compared to diabetic rats.

At the 14th day, administration of the most effective dose (200 mg/kg b.w.) of KH-MβCD showed no significant difference compared to the normal rats. The blood glucose level decreased from 242.10±3.73 to 92.01±3.37 mg/dl after 2 w of treatment with KH-MβCD indicating that using KH in a dose of 200 mg/kg could efficiently decrease the fasting blood glucose levels in diabetic rats, indicating that the hypoglycemic effect is cumulative [28].

Also, the hypoglycemic effect of KH was greater than metformin. Further investigations are required for determination of site (s), cellular and molecular mechanisms of KH pharmacological effect.

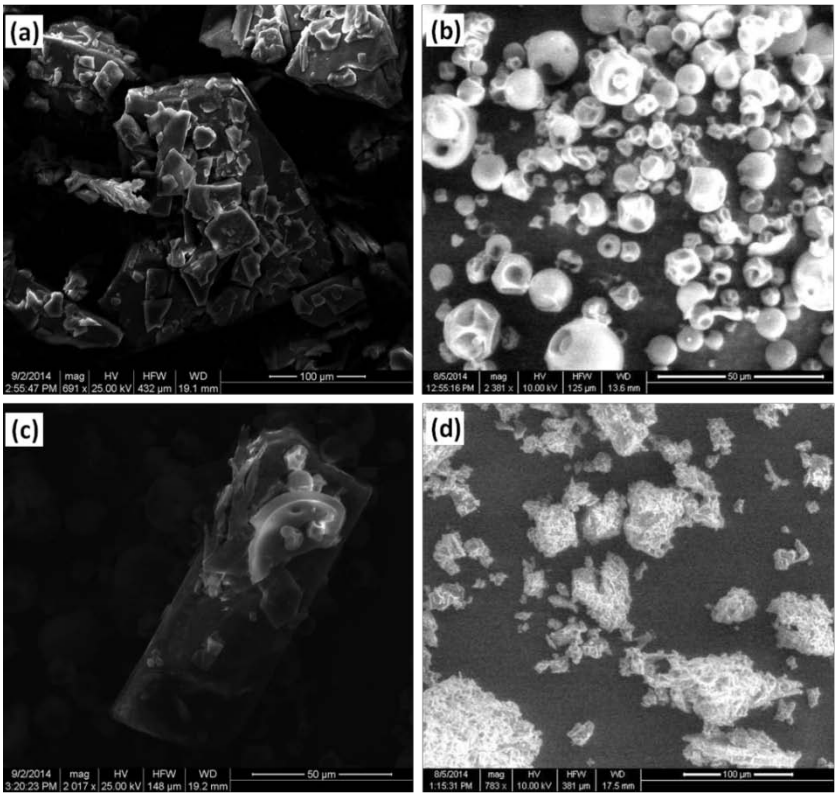


Fig. 6: Scanning electron microscopy of KH (a), MβCD (b), physical mixture (c) and freeze drying (d)

Table 3: Antidiabetic effect of KH-MβCD complexes compared to metformin on fasting blood glucose level of STZ-induced type 2 diabetic rats

Groups	Fasting blood glucose level (mg/dl)±SD		
	3rd day	7th day	14th day
Normal	104.85±4.53*	100.83±0.73*	90.24±2.31*
Diabetic	265.59±3.25@	268.71±1.97@	268.71±2.27@
Metformin	263.23±2.00@	248.19±1.98@	104.76±9.43*@
KH-MβCD complex(100 mg)	239.16±7.75*@	192.56±2.86*@	104.09±11.01*@
KH-MβCD complex(200 mg)	242.10±3.74*@	183.55±6.59*@	92.01±3.37*

Results are expressed as means±SD (n=6). *p<0.05 significant from the diabetic group, @ p<0.05 significant from normal group.

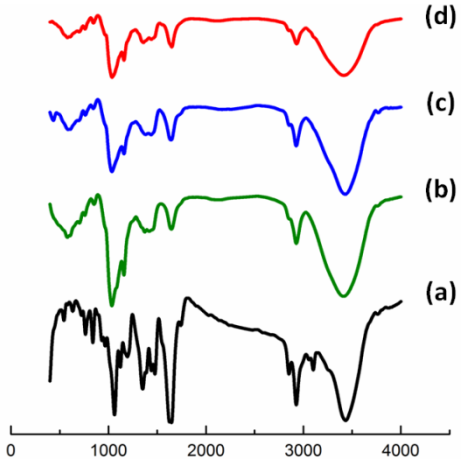


Fig. 7: FTIR spectrum of KH(a), MβCD(b), physical mixture (PM)(c) and freeze drying (FD)(d)

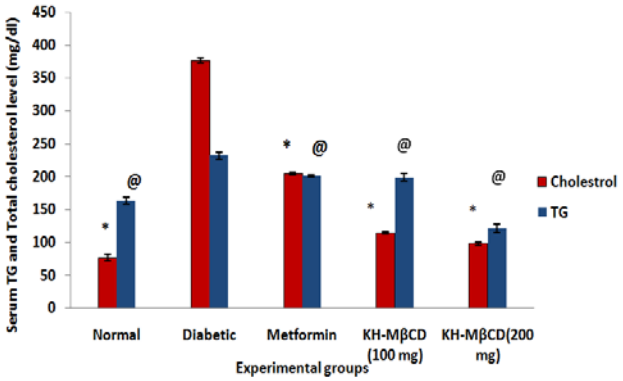


Fig. 8: Effect of KH-MβCD on triglyceride and total cholesterol levels of diabetic rats. Results are expressed as means±SD (n=6).* significance difference from diabetic rats for total cholesterol level and @ significance difference from diabetic rats for triglyceride level (TG)

Hypolipidemic effect of KH in hyperglycemic rats

Elevation of total cholesterol and triglycerides level is one of the characteristics of hyperglycemia as the deficiency in insulin production leads to the failure to activate the enzymes resulting in hypertriglyceridemia and hypercholesterolemia [59]. The change in cholesterol and triglycerides level for normal, diabetic and hyperglycemic rats has been observed for 14 d (fig. 8). The results showed that KH has a valuable effect in improving both cholesterol and triglycerides levels. Treatment of diabetic rats with KH at dose 100 and 200 mg/kg significantly lower the serum triglyceride and total cholesterol levels compared to diabetic control ($p<0.05$). The above-obtained results proved that KH can effectively treat hyperlipidemia in diabetic rats.

Diabetes is a disease that is strongly linked with both microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) and macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke), resulting in organ and tissue damage. The results obtained for hypoglycemic and hypolipidemic effects of KH may prove that KH can act as an active, supportive treatment to resolve these complications indirectly.

CONCLUSION

The present study has demonstrated the feasibility of enhancing the solubility and dissolution rate of KH by formulating inclusion complex with MβCD. An inclusion complex of KH-MβCD was formed as shown in the phase-solubility diagram in the ratio 1:1. The addition of HPMC as water soluble polymer resulted in subsequent improvement of the drug solubility. The solid state characterization; DSC, XRD and FT-IR confirmed the formation of the inclusion complex with complete disappearance of the free drug. The present study also revealed the hypoglycemic effect of the new complex of KH representing a promising supportive treatment in diabetes. The new product will allow a reduction of the oral dose with better control over drug side effects thus optimizing the safety as well as the efficacy of the drug.

CONFLICT OF INTERESTS

The authors who have taken part in this study declared that they don't have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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