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Enhancement of the Dissolution Rate of Ketoconazole Through a Novel Complexation with Humic Acid Extracted from Shilajit

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The objective of the current research work was to investigate the potentiality of humic acid, extracted from shilajit, as a bioavailability enhancer. Ketoconazole was selected as a model drug because of its insolubility in water except at low pH, leading to poor bioavailability. Complexes of humic acid with ketoconazole were prepared in a molar ratio of 1:1 by solvent evaporation, spray drying and physical mixing methods. The prepared complexes were characterized by differential scanning calorimetry (DSC) and X-ray diffractometry (XRD). Phase solubility and intrinsic dissolution rate study of ketoconazole with humic acid were carried out in phosphate buffer of pH 5 and 6 to study the interaction between ketoconazole with humic acid. Phase solubility study indicates that solubility of ketoconazole was significantly increased at humic acid concentration greater than 0.8 % w/v due to micellization of humic acid. DSC and XRD studies showed differences between ketoconazole and ketoconazole-humic acid complexes prepared by various methods. Solvent evaporated and spray dried complexes showed significant improvement in dissolution rate as compared to ketoconazole or ketoconazole-humic acid physical mixture. Further, spray-dried complexes showed better dissolution rate than solvent evaporated complexes. The results indicate that the humic acid extracted from shilajit could be used to increase the dissolution rate and hence the bioavailability of poorly water-soluble drugs.

Key Words: Shilajit, Humic acid, Ketoconazole, Complexation, Dissolution enhancement.

INTRODUCTION

Humic substances are the most important source of organic carbon in the environment. They are product of the decomposition of dead and decaying plant and animal tissues. Their colloidal characteristics and the high surface functionality make them excellent adsorbents, possessing a superior capacity for the retention of ionic and molecular pollutants and for facilitating the processes of mobilization/immobilization of these in the environment. They are mainly fractionated into humic acid, fulvic acid

and humin, as a function of their solubility at different pH values. Fulvic acid is soluble at any pH; humic acid is soluble at alkali pH and humin are insoluble at any pH. The solubility of these fractions is closely related to molecular mass, structural branching complexity, molecular polarity and chemical composition. The structures of humic substances are at present ill defined despite many decades of scientific research. However the structural study so far carried out on humic acid showed that they have open flexible structure having voids in its structure. The porous structure of this acid indicates that they have inner hydrophobic and outer hydrophilic structure and thus have a capability to form a complex with non-polar solute and hydrophobic drug molecules[1]. Humic substances occur ubiquitously in the environment such as in soil, peat, coal, shilajit, *etc*.

The toxicity study report on humic acid showed that it is non-toxic. The Chinese and US Food and Drug administration have approved the use of humic acid for internal and external use. There are also some reports on the use of humic acid as a dietary supplement and for enhancing the bioactivity of the medicinal drug[2,3]. Shilajit is one of the richest source of humic substances and has been used for thousands of years under the indigenous systems of medicine like Ayurveda, Siddha and Unani as a health tonic and rejuvenator[4]. Chemical studies showed that it comprised of humus (60-80 %) along with other components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids. The major physiological action of shilajit was found to be due to the presence of the bioactive dibenzo-α-pyrones along with humic and fulvic acid, which acted as carrier molecules for the active ingredients[5,6].

The structural report on shilajit humic acid showed that it has an open flexible structure having a void in its structure[7]. Humic acid of shilajit has an average molecular weight of 6500. In the present study we have extracted the humic acid from shilajit and was evaluated for its dissolution rate enhancement (bioavailability enhancement) capacity for the poorly water-soluble drug. To study the potential of humic acid as a bioavailability enhancer, ketoconazole was selected as a model drug. Ketoconazole (KET) is an imidazole antifungal agent used in the treatment of candidiasis, blastomycosis and other systemic fungal infections[8]. Ketoconazole is a weak base with pKa of 2.94 and 5.61. It is practically insoluble in water except at pH < 3. The major drawback associated with the therapeutic application of ketoconazole was poor bioavailability upon oral administration because of its very low aqueous solubility and hydrophobic structure[9]. The aim of the present study was to investigate the complexation behavior of humic acid with KET and its effect on solubility and dissolution rate enhancement of KET in aqueous buffer of pH 5.0 and 6.0.

EXPERIMENTAL

Ketoconazole (*cis*-1-acetyl-4[4-[[2-(2,4-dichlorophenyl)-2(1H-imidazol-1-ylmethyl)-1,3 dioxolan-4-yl]methoxy]phenyl]piperazine) was supplied by skylark, (New Delhi, India) and shilajit from Pioneer enterprises (Mumbai, India). All other solvent and materials were of analytical reagent grade.

Analysis of ketoconazole: Ketoconazole was analyzed by method reported by Ene *et al.*[10] with minor modification. Briefly, the samples were injected manually onto Licosorb RP-18 column (125 × 4.6 mm, particle size 5 μ) attached to the 2487 Waters HPLC model. The mobile phase used was water:acetonitrile:diethylamine (50:50:0.05 v/v/v) at a flow rate of 1.5 mL min^{-1}. The detection was carried out using UV detector at 260 nm.

Extraction of humic acids from shilajit: Humic acid was extracted from shilajit as per the method reported by Ghosal *et al.*[11]. Finely powdered shilajit (200 g) was extracted with 500 mL each of hot chloroform, ethyl acetate and methanol to remove the bioactive components. 50 g of the extracted shilajit was then taken and dispersed in 500 mL of 0.1 N sodium hydroxide solution with intermittent shaking in presence of nitrogen at room temperature for 24 h. The suspension was filtered and the filtrate was acidified with dilute hydrochloric acid to a pH of less than 3. The solution was allowed to stand at room temperature overnight leading to separation of humic acid as coagulate, which was filtered, dried and pulverized.

Phase solubility studies: Phase solubility study was carried out as reported by Higuchi and Connors[12]. Excess KET was added to vials containing various concentration of humic acid (0 - 1.4 % w/v) and were shaken in water bath at 25 ºC until equilibrium was reached (7 d). The contents of each vials was filtered (0.22 μm pore size) and the concentration of ketoconazole was measured by validated HPLC method.

Preparation and characterization of solid complexes: The solid complexes of KET-humic acid were prepared at a molar ratio of 1:1 by solvent evaporation, spray drying and physical mixing methods.

Physical mixing method: Physical mixture of KET with humic acid was prepared by pulverizing and thereafter mixing both solids in mortar with pestle.

Solvent evaporation method: KET and humic acid was dissolved in ethanol before solvent evaporation. The solvent was removed using Rotaevaporator (Scientific corporation, New Delhi) under reduced pressure. The solvent evaporated complex was collected from the flask, dried and pulverized. The yield of this process was about 95 %.

Spray drying method: KET and humic acid was dissolved in ethanol and spray drying was carried out in SMST mini spray dryer (SMST corp., Kolkata, India) using the following optimized condition-flow rate: 4 mL/min, outlet temperature: 70ºC, atomizing air pressure: 3 kg cm^{-2}. The yield of the spray drying process was 55 %.

The complexes were characterized by powder X-ray diffractometry (XRD) and Differential scanning calorimety. Powder X-ray diffraction patterns study were carried out with a Philips X-ray diffractometer (Panalytical 1830 BASED) using Cu-Ka radiation.

Thermal analysis was performed using a Perkin Elmer differential scanning calorimeter equipped with a computerized data station (scanning rate 10 ºC min^{-1}).

Dissolution rate studies: Intrinsic dissolution rate study of KET and the prepared complexes was performed in phosphate buffer of pH 5 and 6 as a dissolution medium at 37 ± 0.5 ºC for 3 h. The samples, corresponding to 30 mg of KET, were placed in 100 mL of the dissolution medium and stirred at 200 rpm using Remi magnetic stirrer. The concentration of the drug dissolved at various time intervals was analyzed by HPLC at 260 nm. All dissolution studies were carried out in triplicate.

RESULTS AND DISCUSSION

The extraction of shilajit with solvent of graded polarity *viz.*, chloroform, ethyl acetate and methanol removed the fatty acids, benzoic acid and hydroxy benzoic acid (chloroform soluble), benzo-pyrones, triterpenes and sterols (ethyl acetate soluble) and α-amino acids, ellagic acid, *etc.* (methanol soluble) components[13]. Finally, the acidic treatment of extracted shilajit lead to separation of humic acid, which was collected, dried in oven at 100 ºC for 2 h and pulverized. The yield of humic acid was 8.0 %.

Interaction between KET and humic acid in aqueous media: Phase solubility profile of KET-humic acid is shown in Fig. 1. It was observed that solubility of KET at pH 5 and 6 was increased significantly by humic acid at concentration greater than 0.8 % w/v. Gaffeney *et al.*[14] reported that humic acid extracted from soil forms micelles in aqueous solution at 0.7-0.8 % w/v concentration, which further supports and suggest that the increase in solubility of KET in presence of humic acid is because of micelle formation.

The complexes were characterized by powder X-ray diffractometry and differential scanning calori-metry. The DSC thermogram and XRD spectra of the prepared complex are shown in Figs. 2 and 3, respectively.

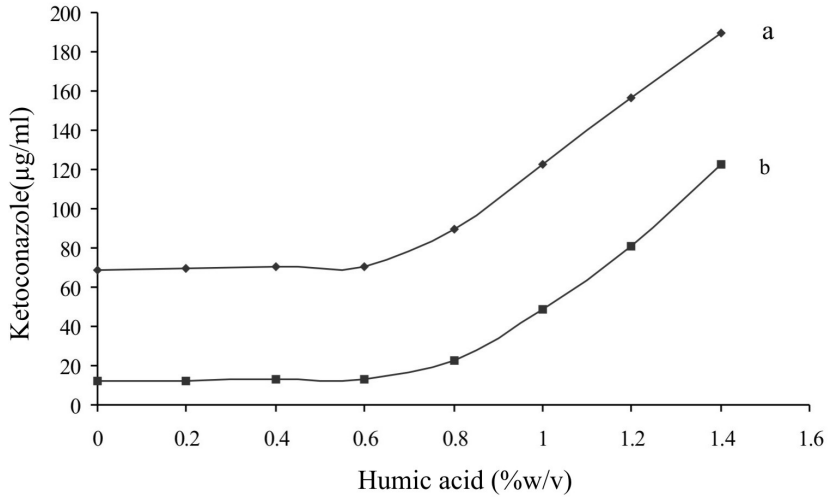

Fig. 1. Phase solubility diagram of ketoconazole and humic acid in buffer of pH = 5 (a) and pH = 6 (b) at 25 ºC

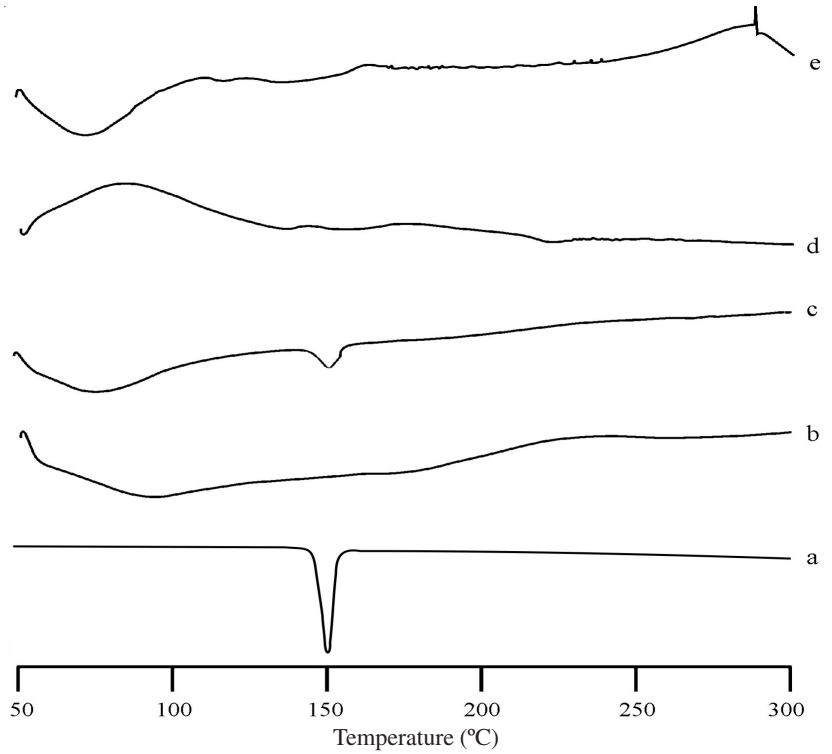

Fig. 2. Differential scanning calorimetry of the different KET-humic acid systems: (a) KET; (b) Humic acid; (c) KET-humic acid (1:1) physical mixture; (d) KET-humic acid (1:1) solvent evaporated complex and (e) KET-humic acid (1:1) spray dried complex

Fig. 2 showed that the DSC thermogram of physical mixture was superimposed as that of humic acid and ketoconazole and showed one endothermic peak at 151 ºC (due to melting point of ketoconazole). However, this peak has disappeared in the complexes prepared by solvent evaporation and spray drying method. This suggests the formation of complex between ketoconazole with humic acid.

Fig. 3 showed that the XRD diffraction spectra of the physical mixture was superimposed as that of ketoconazole and humic acid, while those of spray drying and solvent evaporated complex shows absence of peaks which suggest formation of complex between ketoconazole with humic acid.

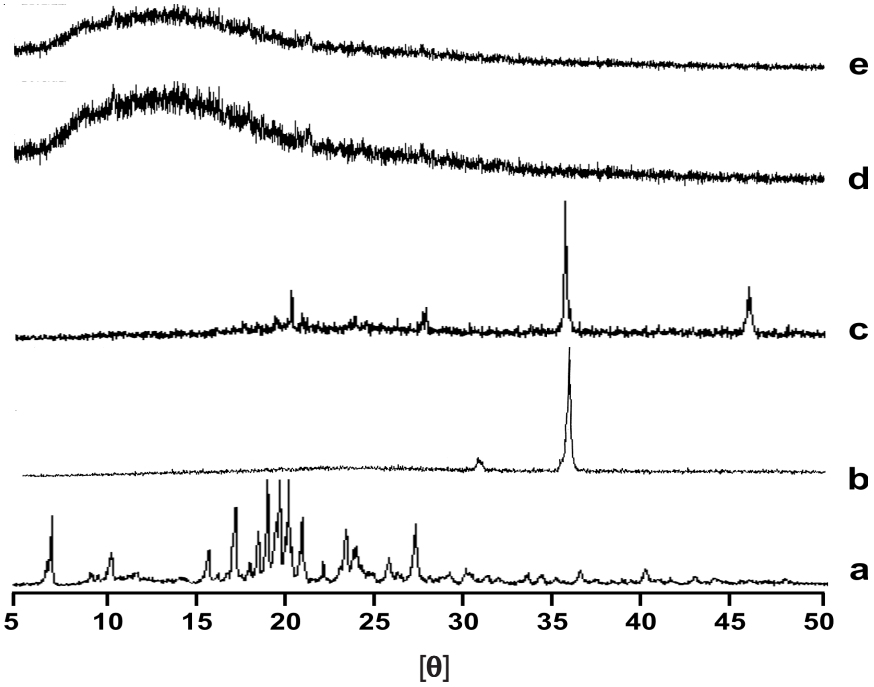

Fig. 3. Powder X- ray diffractogram pattern of the different KET-humic acid systems: (a) KET; (b) Humic acid; (c) KET-humic acid (1:1) physical mixture; (d) KET-humic acid (1:1) solvent evaporated complex and (e) KET-humic acid (1:1) spray dried complex

Effect of complexation on the dissolution of the drug: The dissolution profile of ketoconazole and the ketoconazole-humic acid complexes at pH 5 and 6 are shown in Figs. 4 and 5, respectively. The Sheff's test (F-test) was used for comparing the dissolution efficiency of the prepared formulations at both pH 5 and 6. The Sheff's test grouped the formulation as:

KET; KET-humic acids (1:1) physical mixture; KET-humic acids (1:1) solvent evaporated complex; KET-humic acids (1:1) spray dried complex.

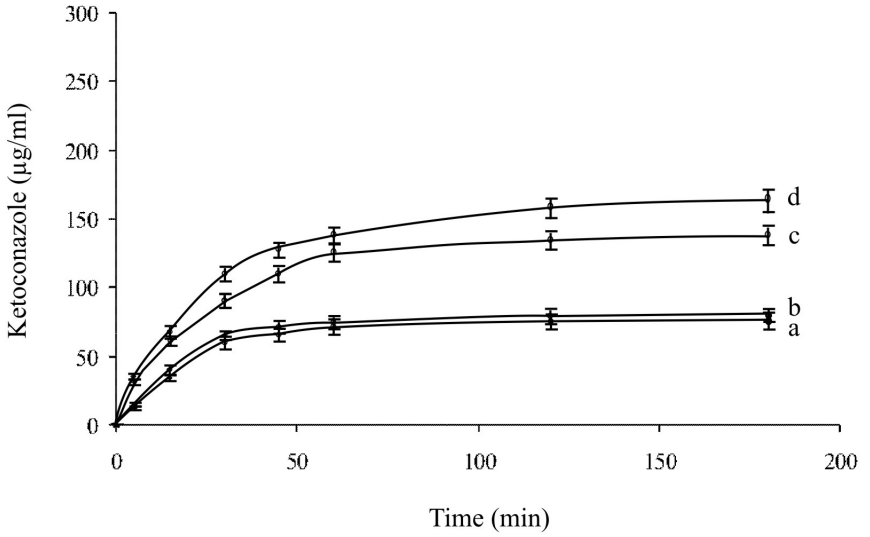

Fig. 4. Dissolution profile of ketoconazole and its complexes at pH 5: (a) KET; (b) KET-humic acid (1:1) physical mixture; (c) KET-humic acid (1:1) solvent evaporated complex; (d) KET-humic acid (1:1) spray dried complex

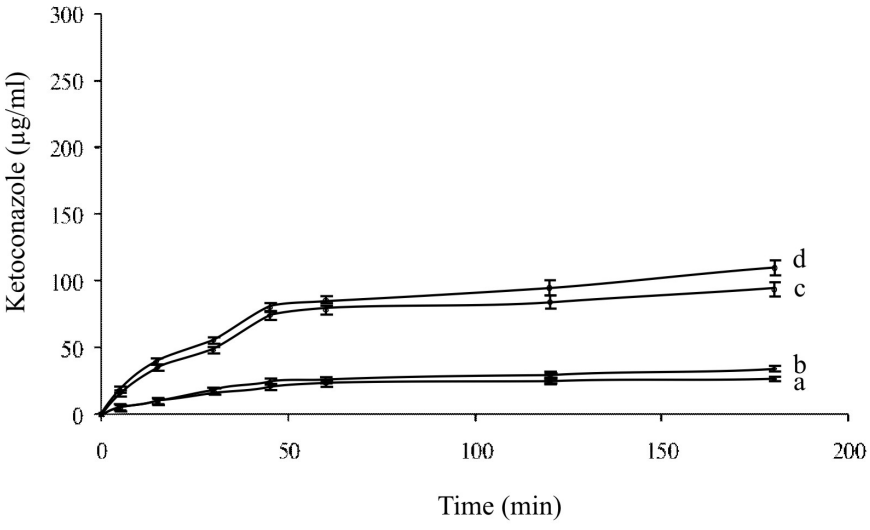

Fig. 5. Dissolution profile of ketoconazole and its complexes at pH 6: (a) KET; (b) KET-humic acid (1:1) physical mixture; (c) KET-humic acid (1:1) solvent evaporated complex; (d) KET-humic acid (1:1) spray dried complex

One-way analysis of variance in dissolution efficiency (0-180) reveals significant difference between the different formulations of KET. The dissolution efficiency for KET-humic acid (1:1) complexes were $F_{(3,24)}$ = 4.47 and $F_{(3,24)}$ = 9.63 for pH 5 and pH 6.0, respectively at a 5 % level of significance.

The F-test calculated values for the KET formulations at 5 % level of significance in both the dissolution media are much higher than tabulated value ($F_{(3,24)}$ = 3.008), which suggest and supports that the dissolution efficiency of the prepared complexes is much higher as compare to KET alone.

In buffer solution pH 6, the dissolution profile was almost the same as that of buffer solution pH 5 except that low amount of drug goes into the aqueous medium. The intrinsic dissolution rate studies at pH 5 and 6 showed that the dissolution of KET from ketoconazole-humic acid physical mixture was almost similar to ketoconazole alone but the complexes prepared by solvent evaporation and spray drying method has exhibited a marked increase in solubility of ketoconazole. Further, the spray dried complex showed better dissolution rate as compare to solvent evaporated complex which may be ascribed to the amorphous nature of the spray dried complex.

Conclusion

Complex of KET-humic acid at a molar ratio of 1:1 can be obtained by spray drying and solvent evaporation method. The intrinsic dissolution rate studies showed that the dissolution of KET from KET-humic acid (1:1) physical mixture was almost similar to KET alone, but the complexes prepared by solvent evaporation and spray drying method exhibited a marked increase in the dissolution rate of KET. Further, the spray dried complexes showed better dissolution rate as compared to solvent evaporated complexes, which may be ascribed to the amorphous nature of the spray dried complex.

The dissolution profiles in buffer solution of pH 5 and 6 are almost similar except that higher dissolution rate was observed in buffer solution of pH 5 which is most probably due to ionization of drug at lower pH. The study proved that humic acid has a potential to increase the dissolution rate of a poorly water-soluble drug(s) and hence could be used as a bioavailability enhancer.

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