Characterization of Protein binding using Terbinafine as model drug through Egg Shell Membrane in Stirred media and Unstirred Media.

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ABSTRACT : The process where a drug binds with the proteins is called protein binding . Protein binding is characterized by association of proteins present inside the body such as HAS, Globulin, Glycoproteins etc. The effect of protein binding on the antifungal property of terbinafine by the action of diffusion using stirred & unstirred method was studied with the help of diffusion studies using UV spectrophotometer. The drug terbinafine follows first pass metabolism that is disturbed by intervention of serum albumin leading to interruption in absorption symmetry and decreases distribution of drug. Binding of the drug to major serum components including albumin and lipoprotein (LDL and HDL) reduces bioavailability. This results in decreased diffusion of terbinafine in experimental models consisting of stirred and unstirred medium.

KEYWORDS : Terbinafine , Protein Binding , Semipermeable (egg shell) Membrane

INTRODUCTION

Terbinafine belongs to allylamine class of antifungal used to treat dermatophytes and candida as well as other fungal infections. It inhibits ergosterol synthesis by epoxidase. Approximately 75% of an oral dose of terbinafine is absorbed. The absolute bioavailability of terbinafine is approximately <50% by first pass metabolism. To study the effect of protein binding on rate of diffusion of terbinafine was studied using two different models.

Drugs entering in the body can interact with a number of tissue components and proteins which are classified into two classes: Blood and extra vascular tissues. Normally the molecule interacts with macro molecules such as proteins DNA or adipose

Binding of drug may affect the target molecules by significant alteration. It may be reversible and irreversible reversible drug binding normally involving the chemical bond like hydrogen bonds, hydrophobic bonds, ionic bonds and Vander Waal's forces whereas irreversible drug binding can be seen when covalent binding occurs.

Diffusion it is the net movement of molecules from are region of high concentration to low concentration under the influence of concentration gradient

UV spectrophotometer method has been used for the determination of the terbinafine in vitro protein binding studies.

MATERIALS AND METHODS

Materials: Terbinafine, egg albumin, egg shell, Conc. hydrochloric acid, Sodium Phosphate Dibasic Heptahydrate, Sodium Phosphate Monobasic Monohydrate.

Apparatus Required: Beaker, Petri dish, measuring cylinder, weighing machine, butter paper, test tubes, test tubes stand.

Methods

Preparation of Dissolution Method (pH – 6)

The diffusion media was prepared by taking 800 ml of distilled water in a suitable container. 3.67 gram of sodium phosphate heptahydrate was added to the solution. Then add 11.91 gram of sodium phosphate monobasic monohydrate to the solution. Adjusted the solution to final desired pH using hydrochloric acid. Lastly distilled water was added until the volume reached to 1000 ml.

Isolation of Diffusion Membrane (egg shell membrane)

A small hole was made on the top of egg shell by using the sharp object. The yolk and albumin of the egg were removed in the petri dish. Then the empty egg shell was isolated and, in the beaker, containing concentrated hydrochloric acid the empty egg shell was completely dipped

It was left for 15 to 20 minutes. After 20 minutes the egg shell was removed from the conc. HCL and put in the beaker filled with water for isolating the egg shell membrane.

Preparation of drug sample

Two solutions are prepared

Solution 1: The drug 50 mg was taken and added to 50 ml of ethanol to attain a concentration of 1 mg/ml. 10 ml of this sample was taken in test tube A as reference or control.

Solution 2: In another test tube B, 5ml of 1mg/ml drug sample was taken and mixed with 5ml of egg albumin (egg white) representing similar properties a s Human Serum Albumin .

Diffusion studies

The required length of egg membrane was cut and tied to the mouth of two broken test tubes namely A and B. In two beakers 500 ml of pH 6.8 was taken was made sure that the level of media in both the compartments were equal . The which amount of pure drug was added to the test tube of the diffusion cell and the study was performed for the duration of 80 minutes . At the predetermined time intervals of 5 minutes , 10 minutes , 15 minutes , 20 minutes , 25 minutes and 30 minutes , sample was collected and same value of media was replenished to maintain the sink condition .

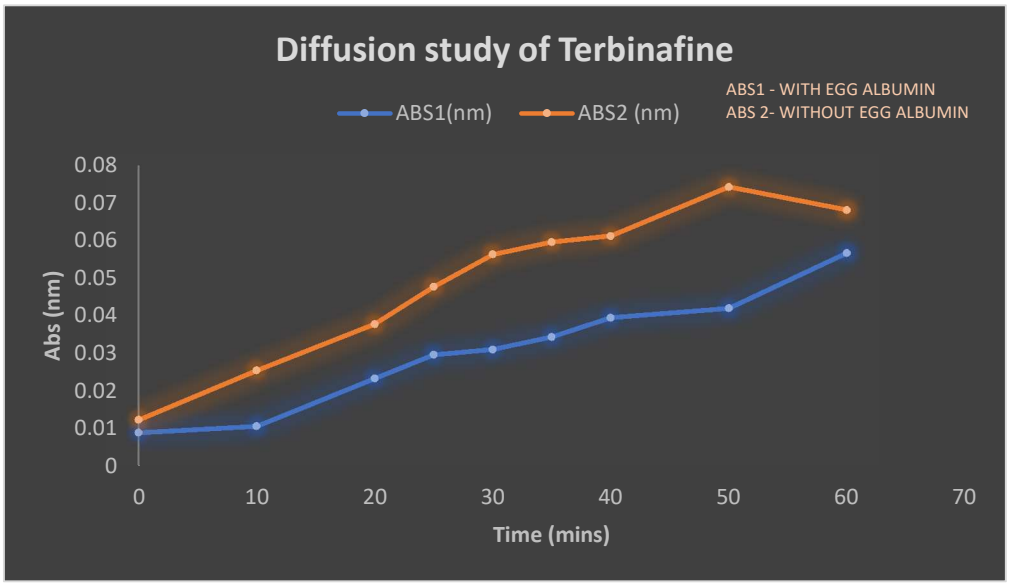
The above procedure was repeated using Stirred medium to ensure the effect of peristalsis and movement of components inside the biological system on Protein Binding.

U.V Spectrophotometric analysis The samples collected from both the mediums at regular intervals was then subjected for U.V spectrophotometric analysis to evaluate the diffusion of drug via semi permeable membrane. The max. wavelength of the drug was found to be 283nm.

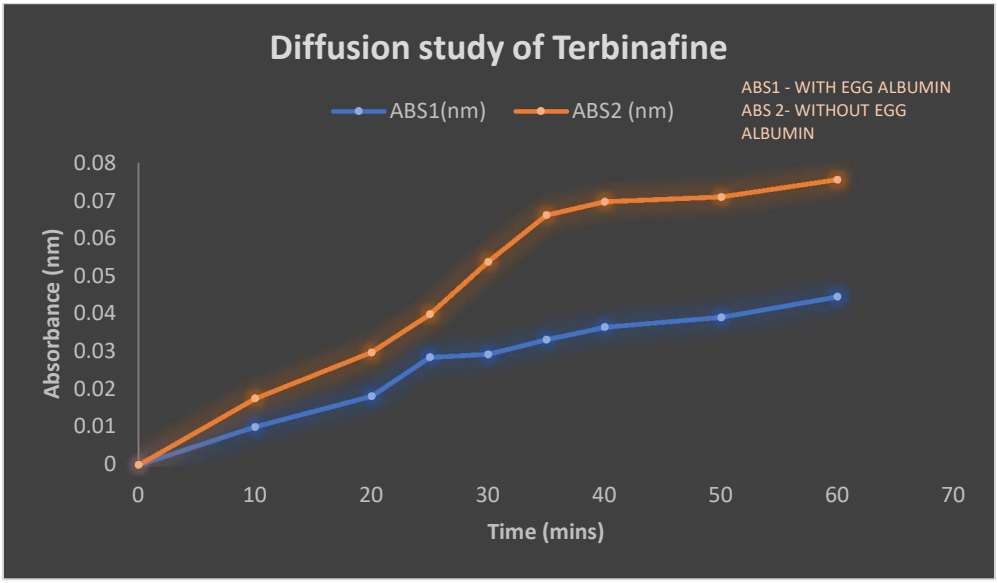
The results are discussed.

RESULTS & DISCUSSIONS:

RESULT: The experiment was successfully performed and as a result it showed high protein binding of the drug Terbinafine .



Graph 1 : Diffusion study of Terbinafine and protein binding in unstirred medium at max. wavelength of 283nm.



Graph 2 : Diffusion study of Terbinafine and protein binding in stirred medium at max. wavelength of 283nm.

Discussion :

Terbinafine , a fungicidal , is an allylamine antifungal drug that is considered to be the first line drug for treating tinea caused by *Trichophyton* infection.

Colour – White

Taste – Metallic

Molecular weight – 328

Physical description – Solid

Melting range – 193 – 198 °C

Bioavailability – Readily absorbed (70-90%)

Metabolism – Liver

Solubility – slightly in water , Soluble in ethanol , Fairly soluble in methanol

CONCLUSION

Antifungal class of drug , Terbinafine an orally and topically active allylamine seen in white , solid with melting range of 193 - 198° C which is slightly soluble in water and freely soluble in methanol possessing high protein binding as observed in the result was the model drug to study characteristics of protein binding through egg shell membrane in stirred and unstirred medium.

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