

Evaluation of Interaction between Ketotifen Fumerate and Theophylline and their Effects on Protein Binding

Mohammed Aktar Sayeed¹, Md. Razibul Habib^{1*}, Md. Mominur Rahman¹,
Mohammed Motaher Hossain Chowdhury² and Md. Sohel Rana²

¹Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

²Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Abstract

The purpose of the present study was to investigate the interactions between ketotifen fumerate and theophylline anhydrous in aqueous media at different pH (2.8 and 7.4). By using Job's continuous-variation analysis and Ardon's spectrophotometric measurement methods the values of the stability constants were determined at a fixed temperature (37 °C) at pH 2.8 and 7.4. *In vitro* study of protein binding was carried out to observe the influence of ketotifen on the protein binding of theophylline by equilibrium dialysis method at pH 7.4. The stability constant values indicated that the formation of complex due to interaction between the drugs were comparatively stable and effective. But when theophylline interacted with ketotifen the values of stability constant was less than 1.00 at pH 7.4 and 3.11 at pH 2.8. The highest percentage binding of ketotifen was 98% and the lowest was 90%. In the presence of theophylline, the highest and lowest values were 90% and 85%, respectively. If given concurrently, ketotifen and theophylline might form stable complex and hence reduce the pharmacological activities of both drugs.

Keywords: Interaction, Stability constant, Job's method, Ardon's method, Ketotifen fumerate, Theophylline anhydrous, Protein binding.

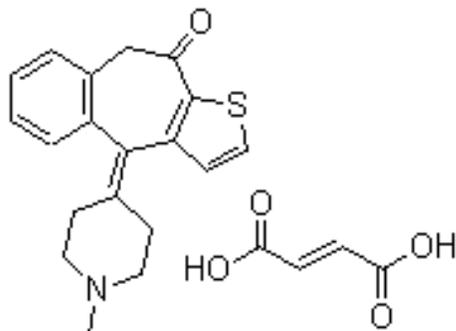
Introduction

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties (Martin and Romer, 1978). It has been demonstrated that it can block *in vitro* release of mediators from rat peritoneal mast cells (Martin and Romer, 1978). Ketotifen has been shown to inhibit the release of histamine and leukotriene from basophil and lung tissue, to antagonize histamine at H₁ receptors, to inhibit calcium uptake, to block the passive cutaneous anaphylactic reaction, to reverse isoprenaline-induced beta adrenoceptor tachyphylaxis, and to inhibit both allergen-induced and drug-induced asthma (Craps *et al.*, 1978). A number of clinical trials of ketotifen have shown it to have a beneficial effect in the treatment of asthma (Hoshino *et al.*, 1998; Tinkelman *et al.*, 1985) equivalent to that of disodium cromoglycate, which has an established place in the treatment of asthma (Brompton Hospital 1972; Clarke and May, 1980). Histamine H₁-receptor blocking drug, ketotifen, which is useful in the treatment of hay fever and asthma, has been found to inhibit anaphylactic histamine

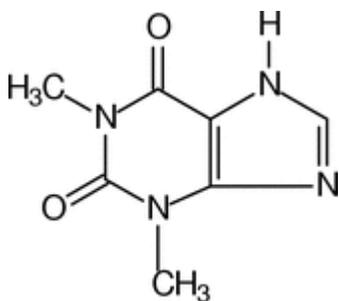
release from animal tissues (Clarke and May, 1980). Theophylline, has bronchodilator properties and is used in the treatment of asthma and chronic obstructive pulmonary diseases. Moreover, theophylline has been shown to have some anti-inflammatory activities, inhibiting the activity of CD4 lymphocytes *in vitro* and mediator release from mast cells (Salamzadeh *et al.*, 2008). It can also inhibit bronchoconstriction produced by exercise and challenge testing. Theophylline has also been shown to have beneficial effects on contraction of diaphragm, an effect which may be particularly useful in patients with chronic obstructive pulmonary diseases (Kidney *et al.*, 1995; Mak, 1997). Drug-drug interactions occur when one therapeutic agent alters either the concentration (pharmacokinetic interactions) or the biological effect of another agent (pharmacodynamic interactions) (Leucuta *et al.*, 2006). The clinical significance of a specific drug-drug interaction depends on the degree of accumulation of the substrate and the therapeutic window of the substrate (Bachmann and Lewis, 2005). The combination of theophylline and

* Correspondence to: Md. Razibul Habib, Department of Pharmacy, International Islamic University Chittagong, Bangladesh.
E-mail: mrhjewel@gmail.com

ketotifen is widely used and some suggest the combination is effective (Benjamin *et al.*, 1994) though others suggest the combination may be embryotoxic, with growth retardation, morphological abnormalities, etc (Bechter and SchÖn, 1988).



Ketotifen fumarate



Theophylline anhydrous

Materials and Methods

Materials

Drugs and chemicals: Ketotifen fumarate and theophylline anhydrous were kind gift from Square Pharmaceuticals Ltd., Dhaka, Bangladesh and were used without further purification. Bovine serum albumin (fraction V) and semipermeable membrane (Medicell, England) were purchased from BDH (England). Sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate, used for the preparation of buffer solutions were purchased from Merck, Germany. Potassium chloride, sodium hydroxide, potassium hydroxide etc. were all of reagent grade.

Equipments: For the experiment we used UV-Visible spectrometer (Model No. UV-1600, Shimadzu, Japan), pH meter (Mettler Toledo, Switzerland), four digit balance

(Mettler Toledo, Switzerland), thermostat water bath (Shimadzu, Japan). A Dunbuff metabolic shaking incubator (Nickel, Electrical Company, England) was used to shake the plasma drug mixtures for the attainment of the equilibrium.

Methods

Preparation of standard solutions: Stock solutions of ketotifen fumarate and theophylline anhydrous were prepared by dissolving them in distilled water. These stock solutions were diluted to desired strengths by buffer solutions to get the working standard solution.

Absorption spectrum analysis: In observation of the spectra, the absorption characteristics of ketotifen fumarate and theophylline and their 1:1, 1:2 and 2:1 mixtures in the solutions of buffers (Mohiuddin *et al.*, 2009) at pH 2.8 and 7.4 were compared with those of each interacting species. The concentration ($1 \times 10^{-5} \text{M}$) of the sample was kept at very dilute levels and the measurements were made using an UV spectrophotometer with a constant temperature cell compartment and automatic recording unit. The stock solutions of the samples were diluted to appropriate levels, with buffers at the desired pH and the spectra were recorded between 190-400 nm. The spectra were compared with the pure sample in each case.

Job's spectrophotometric method (Job, 1928): According to Job's method a series of solutions were prepared in which the concentration of one reactant (usually the cation) was held constant while that of the other was varied. Absorbance of series of ketotifen fumarate with theophylline in different molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were measured by keeping the total mole constant. The observed absorbance of the mixtures at various mole fractions was subtracted from sum of the values for free drugs (ketotifen fumarate and theophylline anhydrous). The absorbance difference (D) was then plotted against the mole fractions of the drug in the mixtures. If the formation constant was reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex were obtained.

Ardon's spectrometric method (Ardon, 1971): The theophylline anhydrous concentration was kept fixed ($2 \times 10^{-4} \text{M}$) while the ketotifen concentrations were varied.

The absorbance of free drug solutions and those of mixtures were measured at the λ_{max} 300 nm at different pHs. From Ardon's equation the values of $1/(D-C\epsilon)$ versus $1/Drug$ were plotted and the values of stability constants were calculated from intercept /slope of the straight lines obtained. In the above equation D is the absorbance of the mixture, C is the molar concentration of ketotifen, ϵ is the molar extinction coefficient of the complex.

Equilibrium dialysis method: The membrane was activated by digestion with 1 M $NaHCO_3$ at 70°C for 4 hours and washed with de-ionised water and immersed in 0.067 M phosphate buffer at pH 7.4. Activated membrane (4 ml capacity) were filled with solutions of protein with different concentration of drugs and their mixtures and immersed in 60 ml Of phosphate buffer and then shaken gently for 6 hours in a metabolic shaking incubator at 37°C. After completion of dialysis the absorbance of buffer was measured at 300 nm and the concentration of bound and unbound drugs were calculated.

Calculation of percentage of protein binding: The percentage of protein binding (F) can be calculated by the following equation:

$$F = \left\{ \frac{[B] - [A]}{[B]} \right\} \times 100$$

where, [A] = molar concentration of free drug in buffer compartment

[B] = molar concentration of drug in plasma compartment.

Statistical analysis: The results were expressed as mean \pm SEM values. Differences in mean values between

experimental data were analyzed by unpaired t test. The probability values less than 0.05 ($p < 0.05$) was defined to be significant.

Results and Discussion

In spectral observation, each of the drug studied showed absorption in UV region. The molecular species of ketotifen fumarate and theophylline when separately mixed showed some changes in absorption characteristics of this drug molecule including some shifts in the absorption maxima. The curves obtained by the Job's method show breaks at different molar concentrations for both drugs. It was found that the curves obtained at pH 7.4 were somewhat flat related to at pH 2.8 (Figure 1).

Continuous variation plot gives information on the relative affinities of the complexes and it also depends on the intrinsic spectral characteristics of each complex. The Ardon's plots have been used to evaluate the stability constants and it has been observed that when values of $1/(D-C\epsilon_A)$ are plotted against $1/Drug$ (Figure 2), good straight lines are obtained obeying the Ardon's equation.

The values of stability constants were 9.78 and 8.02 at pH 2.8 and 7.4, respectively when ketotifen showed interaction with theophylline, whereas the values of stability constants were 3.11 and 0.02 at pH 2.8 and 7.4 respectively when theophylline showed interaction with ketotifen.

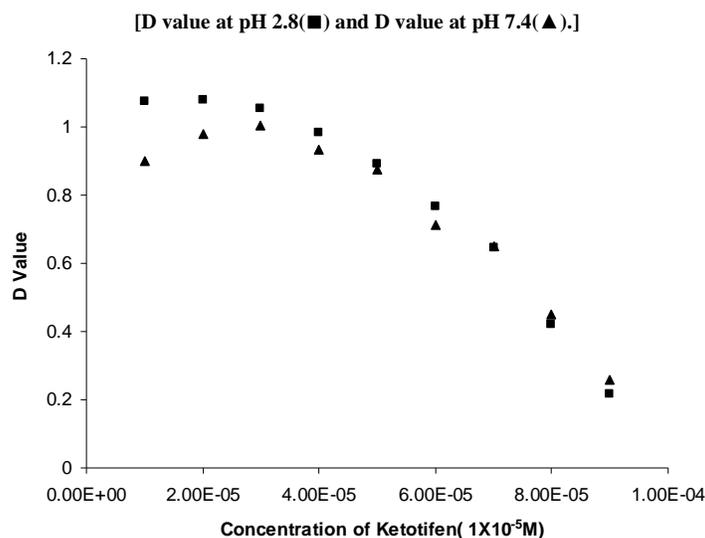


Figure 1. Job's plot for complexation of ketotifen with theophylline.

Table 1. Values of stability constants at different pH

System	pH	Stability constants
Interaction of ketotifen with theophylline	2.8	9.78
	7.4	8.02
Interaction of theophylline with ketotifen	2.8	3.11
	7.4	0.02

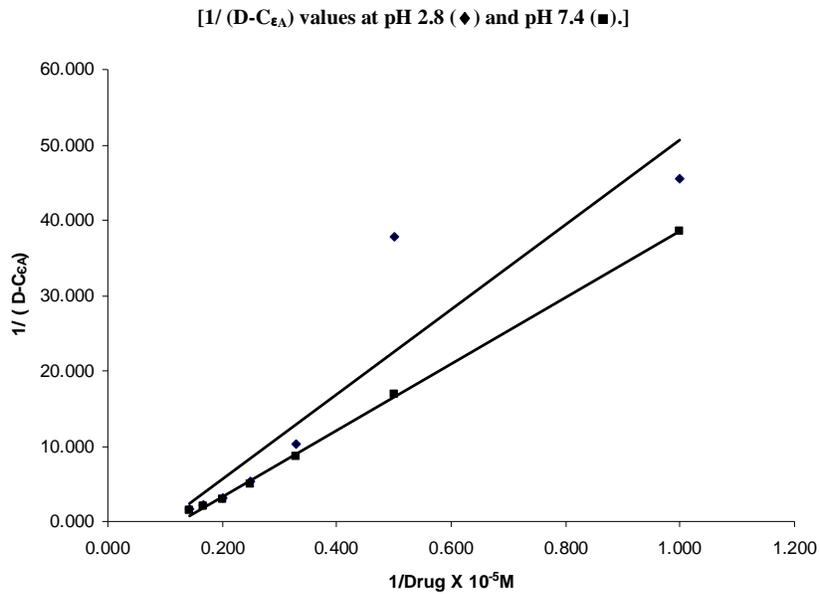


Figure 2. Ardon's plot for complexation of ketotifen with theophylline

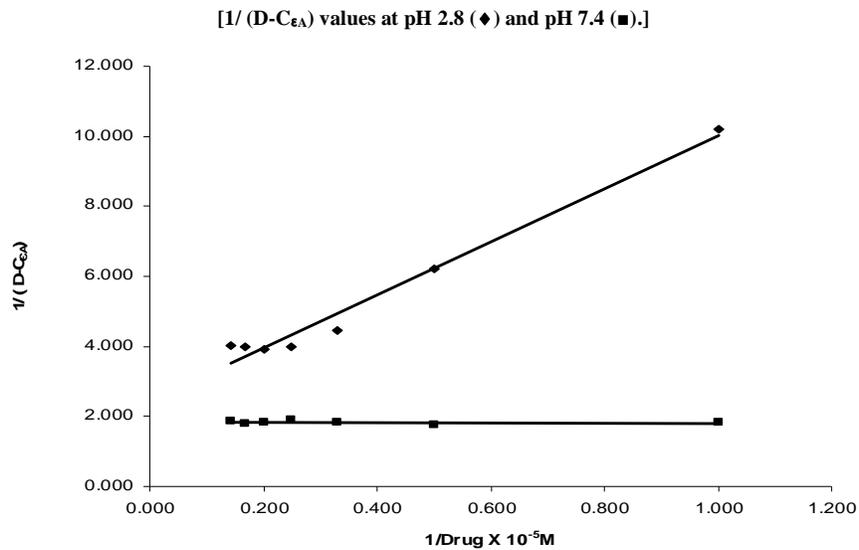


Figure 3. Ardon's plot for complexation of theophylline with ketotifen

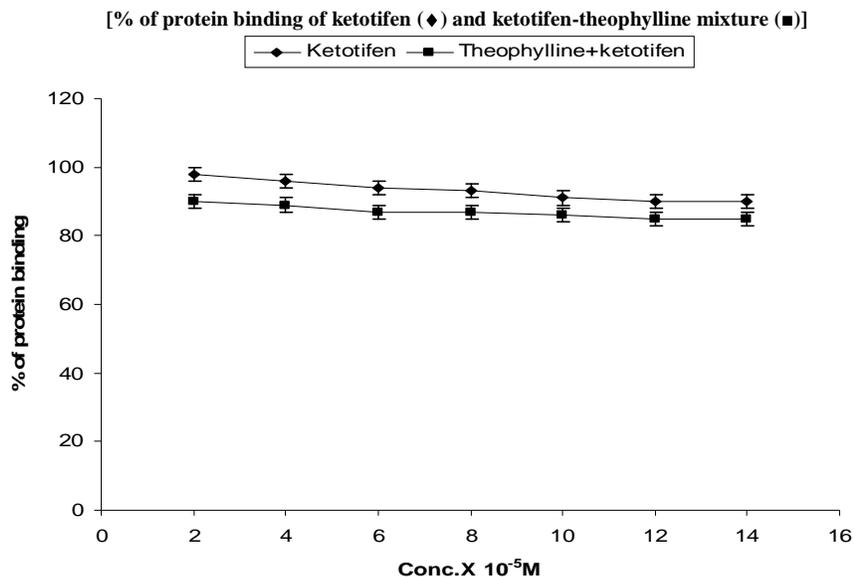


Figure 4. The pro tein binding of ketotifen and theophylline at pH 7.4

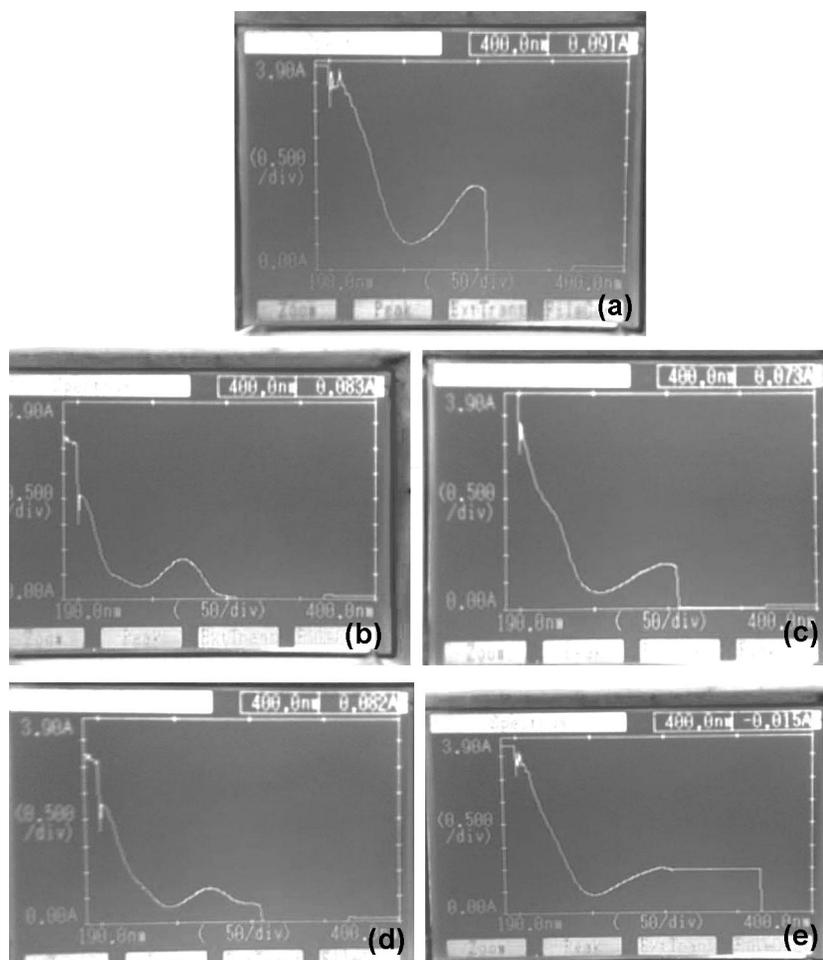


Figure 5. Spectra of ketotifen and their complexes at pH 2.8. (a) Ketotifen fumarate, (b) Theophylline, (c) Ketotifen:Theophylline=1:1, (d) Ketotifen:Theophylline=1:2, (e) Ketotifen:Theophylline=2:1.

In figure 4, the percent of protein binding of ketotifen fumarate and theophylline anhydrous with ketotifen at pH 7.4 (at 37 °C) showed significant results. The data shown as mean \pm SEM, indicates significant change ($p = 0.01$) in protein binding.

The *in vitro* determination of percentage of protein binding of ketotifen fumarate and its 1:1 mixture of

theophylline anhydrous was conducted at 37 °C and at a pH of 7.4. The highest percentage of protein binding of ketotifen with bovine serum albumin was found to be 98% and the lowest was 90%. In the presence of theophylline, the highest and lowest value was 89% and 83%, respectively.

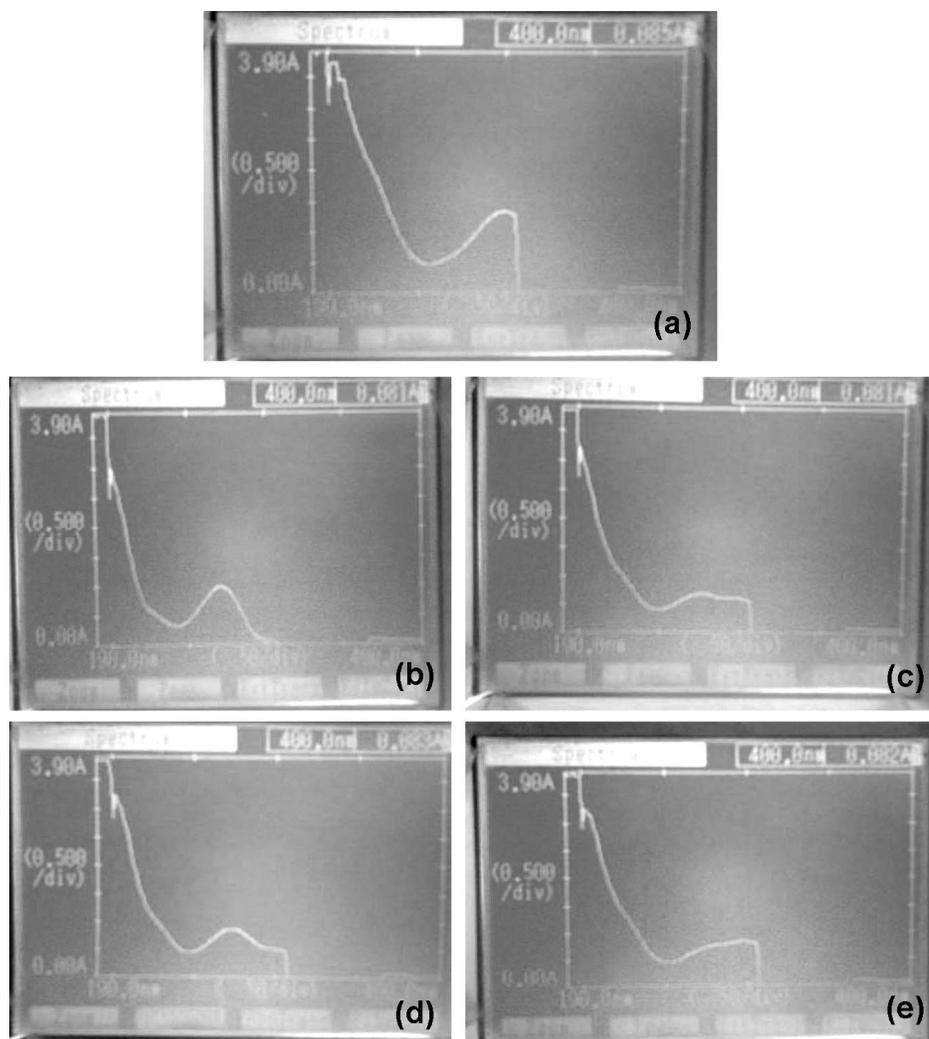


Figure 6. Spectra of ketotifen and complexes at pH 7.4. (a) Ketotifen fumarate, (b) Theophylline, (c) Ketotifen:Theophylline=1:1, (d) Ketotifen:Theophylline=1:2, (e) Ketotifen:Theophylline=2:1.

Initial detection of complexation of ketotifen fumarate with theophylline was done from the nature of spectra of pure compounds as well as their 1:1, 1:2 and 2:1 mixtures in buffer solution of pH 2.8 and 7.4 at a fixed concentration (1×10^{-5} M). It is obvious that each

compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultra-violet radiation. For this reason the spectrum of any pure compound obtained from UV-spectrum would be of one kind which would be totally

different from the other compounds or the complex of that compound with other compounds. The spectra of ketotifen fumarate at different pH showed a sharp absorption maximum at 300 nm. When theophylline is mixed with ketotifen in 2:1 ratio the intensity of the peak of ketotifen had changed remarkably (absorbance decreases) i.e. absorption characteristics were altered due to interaction but the position of the compounds did not shift at pH 2.8. But when theophylline was mixed with ketotifen in 2:1 ratio at pH 7.4, the intensity of the peak of ketotifen had changed remarkably (absorbance increases). On the other hand, the UV spectra of ketotifen were unchanged in 1:1, 1:2 and 2:1 mixture of ketotifen fumarate with theophylline at pH 2.8 and 7.4. Very low stability constant values (between negative values and 1) mean that the formation of complex due to interaction among the drugs is readily dissociated. Again, the values of the resulting stability constant were 9.78 at pH 2.8 and 8.02 at pH 7.4 when complexation occurred between the ketotifen and theophylline. These values are the indication of good interaction between ketotifen with theophylline. It can be assumed that these two drugs cannot safely be administered orally at a time. Following the Ardon's method where theophylline is considered as the parent drug interaction with ketotifen showed a lower stability constant values which indicate the easy solubility of both drugs and minimum drug-drug interaction (Figure 3). The percentage of protein binding of ketotifen was decreased with increased concentration of theophylline anhydrous which attained a steady plateau state when the free drug concentration was around $5 \times 10^{-5} \text{M}$ (Figure 4). On the other hand, theophylline anhydrous was found significantly decreased (Mean \pm SEM, significant because $p=0.01$) when the percentage of protein binding of ketotifen fumarate was also increased but the attainment of steady plateau condition remained unchanged.

Conclusion

The experimental data indicates that interaction of ketotifen with theophylline decrease the free drug concentration of both drugs which results in decrease affinity towards the receptors. Ultimately one or both drugs may show diminished pharmacologic activity. It was observed that ketotifen fumarate and theophylline anhydrous lowered the affinity of protein binding of theophylline, and hence an increase in volume of

distribution of theophylline might have occurred. Therefore it can be inferred that cautions should be exercised during administration of both drugs, although a detail *in vivo* experiment would be necessary to get a clear idea about the therapeutic properties of both drugs.

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