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IN - VIVO STUDY ON INTERACTION OF KETOTIFEN FUMERATE WITH THEOPHYLINE ALONG WITH THEIR IR STUDIES

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ABSTRACT: This is very much important than any other time or ever to know about the medicines we take. If we take several different medicines, suffering from various types of diseases, consult more than one doctor or have certain health conditions, we and our doctors must be aware of all the medicines we take to avoid potential problems, such as drug interactions. The study of Drug interaction between various drugs has been conducted for many years and successful results have also been established. In the continuation of the study of drug interaction we have studied the in vivo interaction between the Kitotifen fumerate and Theophylline along with their IR studies. In-vivo study showed decreased in the plasma concentration of free ketotifen fumarate when given concurrently with Theophylline which may be due to the drug interaction. IR study confirmed that there is an interaction between Ketotifen fumarate and Theophylline with the presence of extra peaks in their combination (aqueous extract 763.84 cm^{-1}, 1595.2 cm^{-1}, 2281.89 cm^{-1},2625.23 cm^{-1}, 2729.39 cm^{-1},3384.25 cm^{-1} & chloroform extract 703.08 cm^{-1}, 2115.04 cm^{-1}, 2161.33 cm^{-1}, 2232.7 cm^{-1}, 2657.06 cm^{-1}, 2701.42 cm^{-1}) forms as compared to that of their pure forms. So, we can say that if both ketotifen fumerate with Theophylline administer concurrently, mild complex can be formed after reaction which can ultimately reduce the pharmacological activities of both combinations of drugs.

INTRODUCTION:

It is often necessary to take more than one drugs at a time and some degree of drug-drug interaction occurs with concomitant use of drugs. Drug interactions can cause serious harm to the patient, sometimes causes serious adverse reactions and now a days become an obvious concern for the health care providers. For example, drug interactions, particularly with drugs having a narrow therapeutic range, may have serious

adverse consequences. There are various types of drug interactions such as Drug-drug interactions, Drug-food/beverage interactions i.e. Interactions Resulting from Alterations in Gastrointestinal Absorption, Interactions Resulting from Alterations in Metabolizing Enzymes (Enzyme induction, Enzyme inhibition), Interactions Resulting from Alterations in Protein Binding, Interactions Resulting from Changes in Renal Excretion.

- Drug interactions are very common. There are several reasons:
- Health care providers may not be aware of interactions with drugs they prescribe.
- Several health care professionals may prescribe medications for one patient
- Aging patients have multiple health issues and take many medications
- Drug interactions may not be identified as the cause of unexpected treatment results or side effects
- Health care providers may not know about all medications and supplements their patients are taking.

Ketotifen is a benzocycloheptathiophene derivative that has been shown to possess anti-histaminic and anti-anaphylactic properties[1]. It has been demonstrated that it can block in vitro release of mediators from rat peritoneal mast cells[1]. The drug inhibits the release of histamine and leukotriene from basophil and lung tissue, antagonizes histamine at H_1 receptors, inhibits calcium uptake, blocks passive cutaneous anaphylactic reaction, reverses isoprenaline induced beta-adrenoceptor tachyphylaxis, and inhibits both allergen-induced and druginduced asthma[2]. A number of clinical trials of ketotifen have shown it to have a beneficial effect in the treatment of asthma[3,4] equivalent to that of disodium cromoglycate, which has an established place in the treatment of asthma[5]. Ketotifen, which is useful in the treatment of hay fever and asthma, have been found to inhibit anaphylactic histamine release from animal tissues[6].

Theophylline are bronchodilators which relax and open the air passages to the lungs to increase the flow of air through them, and thus are used in the treatment and or prevention of symptoms of bronchial asthma and of reversible bronchospasm associated with chronic bronchitis and emphysema. They are used as muscle relaxants, central nervous system and cardiac muscle stimulants. It relaxes and opens air passages in the lungs, making it easier to breathe.

In a research study was undertaken to investigate the effect of theophylline on circulating vitamin B_6 levels in children with asthma. Steady state serum theophylline and vitamin B_6 [pyridoxal 5'-phosphate (PLP) and pyridoxal (PL)] levels were evaluated in these patients. we conclude that theophylline induces a depression of circulating PLP levels in asthmatic children.[7] Another research result shows since depressed vitamin B_6 plasma levels can occur in patients receiving theophylline, we explored a B_6-theophylline interaction in a rabbit model. B_6 deficiency may contribute to chronic theophylline toxicity; however, pyridoxine administration in the dosage used may not prevent toxicity.[8] Another research result explain on Evaluation of Interaction between Ketotifen Fumerate and Theophylline and their Effects on Protein Binding that concluded if given concurrently, ketotifen and theophylline might form stable complex and hence reduce the pharmacological activities of both drugs.[9]

Objective of this study

- The prime object of this project was to elucidate the possible importance of drug-drug interactions (DDIs) as a contributing factor towards drug safety.
- The main focus of the project was to identify whether there is any interaction between Ketotifen fumerate (antihistamine) with Theophylline (Bronchodilator).The purpose of the present study was to investigate the in–vitro and in-vivo complexation and strength of complexes, which may be formed due to

interaction of Ketotifen fumerate (antihistamine) with Theophylline. The specific purpose was to observe and determine the stability of the complexes, which could be formed between Ketotifen fumerate (antihistamine) with Theophylline. To see the potentiation and attenuation of activity of Ketotifen Fumerate under this condition. Such a study can possibly open up a new avenue to formulate a new dosage form of the drug chosen, as well as developing better combination system of therapy in the area of their need.

- Polypharmacy (prescribing many drugs at a time) is a common practice in case of patients undergoing a major operation, hospitalized patients, and also in geriatric patients. In our in vitro and in-vivo study it was found that Ketotifen Fumerate formed complexes with Theophylline along with some intermediary complexes at room temperature and in isotonic environment.

Materials and Method

Materials

All the chemicals and reagents used in this study were of analytical grade and were stored under optimum storage conditions. The experimental mixtures and solutions were prepared in standard volumetric flasks about one hour prior to recording the data.

Drugs and chemicals

Ketotifen fumerate and Theophylline were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh as a token gift and were used without further purification. Sodium di-hydrogen 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 analytical grade.

Equipments

UV-Visible spectrometer (UV mini-1240, Shimadzu, Japan), pH meter (Cyberscan 500, Hanna, Portugal), Electronic balance (Shimadzu Corporation, Japan), a thermos tatted water bath (Memmert, German) and IR Spectrophotometer (IR Affinity-1 A213747 Shimadzu Corporation, Japan) were used for the test.

The λ_{max} value of drug used in the study

The wavelength for maximum absorption value of the drug used in the experiment is given in the following table 1:

Table 1: The λ_{max} value of drug used in the study

Name	Wavelength (λ_{max})
Ketotifen fumerate	300nm
Theophyline	272nm

Animals for In- Vivo Study

Mice (15-32gm) of either sex bred were used. The animals were housed under standard conditions, maintained on a 12-h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1h before the experiments. Experiment was conducted during the light period.

Preparation of drugs solutions

20ml of each drug solutions were prepared according to their corresponding doses.

Doses: Ketotifen fumarate: 0.1mg/kg

Theophylline: 150mg/kg

Methodology

For Ketotifen fumarate + Theophylline

48 fresh mice were separated for the experiment which were kept in fasting condition overnight for 12hrs. Mice were divided into four groups, 12 mice in each group.

Group I

They were kept as control group for the experiment. They were administered normal saline 0.4ml p.o each and the time was noted as 0min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 300 nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

Group II

They were administered ketotifen fumarate solution 0.4ml p.o each and the time was noted as 0 min. 3 mice were sacrificed after 30 mins, blood sample were collected and their absorbances were taken at 300nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

Group III

They were administered Theophylline solution 0.4ml p.o each and the time was noted as 0 min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 272nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

Group IV

They were administered mixture of ketotifen fumarate + Theophylline solution (1:1) 0.4ml p.o each and the time was noted as 0min. 3 mice were sacrificed after 30mins, blood sample were collected and their absorbances were taken at 300nm and 272nm. Similarly every 3 mice were sacrificed after 60mins, 120mins & 180mins and the same procedure were followed as above.

For Infrared Analysis

The interaction between the drug were analysed by IR spectrum analyses

For analysis of drug interaction between Ketotifen fumarate & Theophylline

- 100mg of pure powder of Ketotifen fumarate was dissolved in 10ml of distilled water in a 50ml beaker, similarly 100mg of pure powder of was dissolved in another beaker
- Both the above solutions were mixed in a separate 100ml beaker with constant stirring
- Then the mixture was transferred to the separating funnel
- The interacted drug was extracted in chloroform.
- Chloroform was evaporated and the precipitated solid drug product was analysed by IR spectrophotometer
- Another experiment for aqueous extract of interacted drug product was also analysed by IR spectrophotometer

Result & Discussion

In-Vivo Study Ketotifen fumarate and Theophylline

- Group I (control group i.e no drug used)

Absorbances at 300nm	30min	60min	120min	180min
	0.064±0.134	0.0663±0.006	0.077±0.006	0.3623±0.263

- Group II (Ketotifen fumarate)

Absorbances at 300nm	30min	60min	120min	180min
	1.143±0.027	0.852±0.031	0.7193±0.018	0.788±0.018

- Group III (Theophylline)

Absorbances at 284nm	30min	60min	120min	180min
	0.263±0.014	0.261±0.007	0.266±0.004	0.521±0.021

- Group IV (Ketotifen fumarate+ Theophylline)

Absorbances at 300nm	30min	60min	120min	180min
	0.51±0.016	0.157±0.075	0.137±0.026	0.178±0.007

Absorbances at 272nm	30min	60min	120min	180min
	0.261±0.007	0.132±0.031	0.123±0.009	0.121±0.021

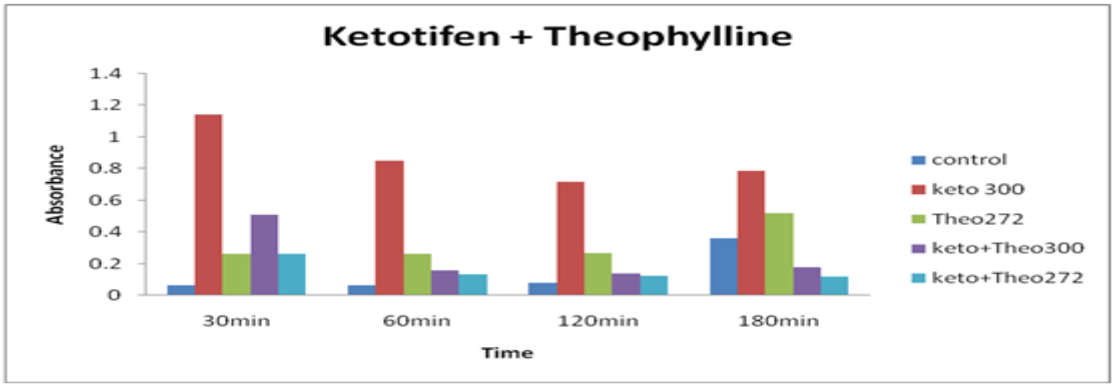


Fig. 1: Graph for Ketotifen fumarate + Theophylline interaction

Infrared Study:

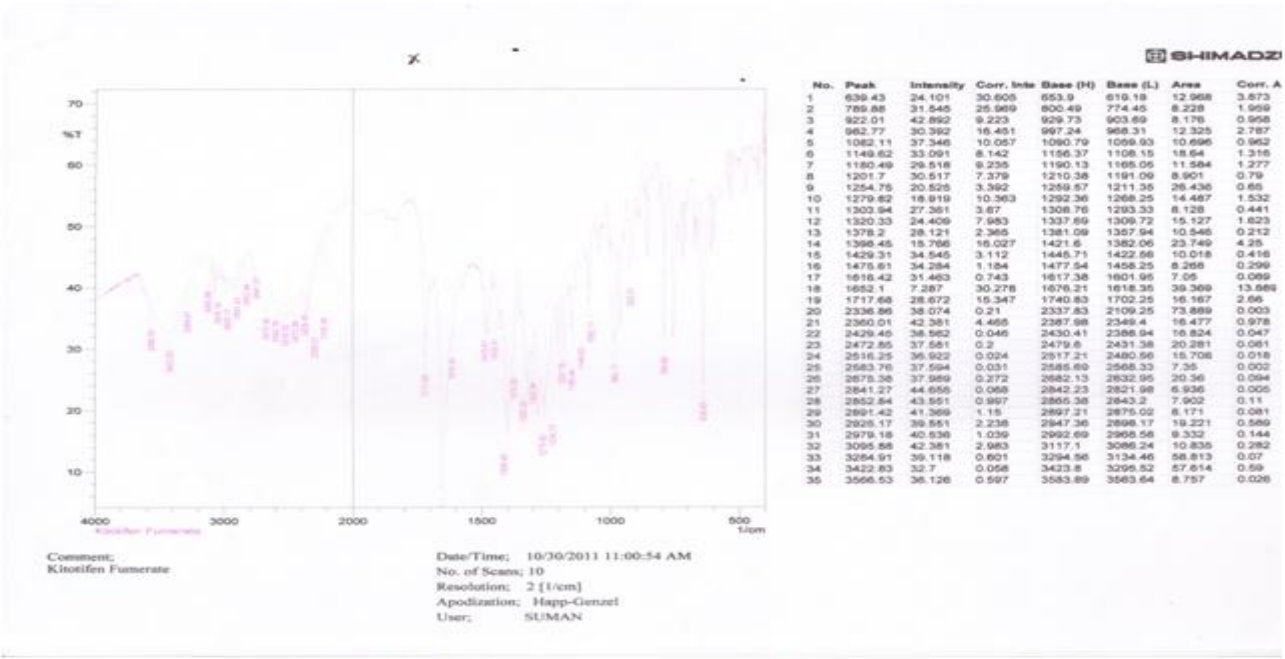


Fig. 2: IR Spectrum of Ketotifen Fumarate

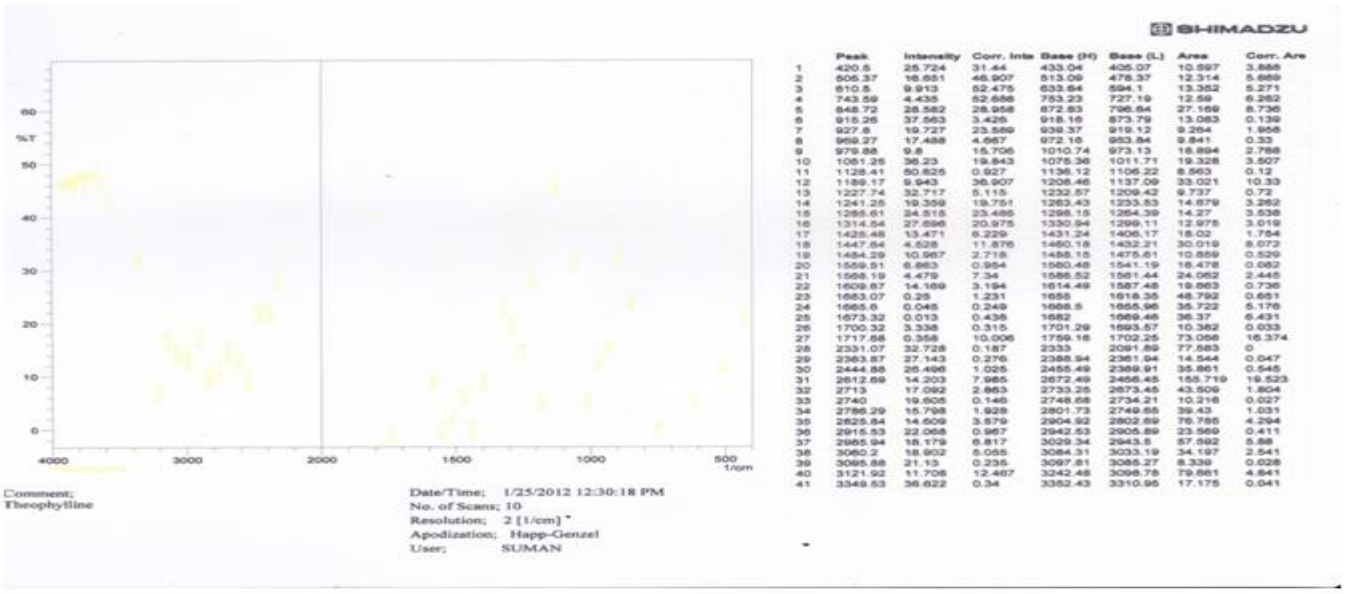
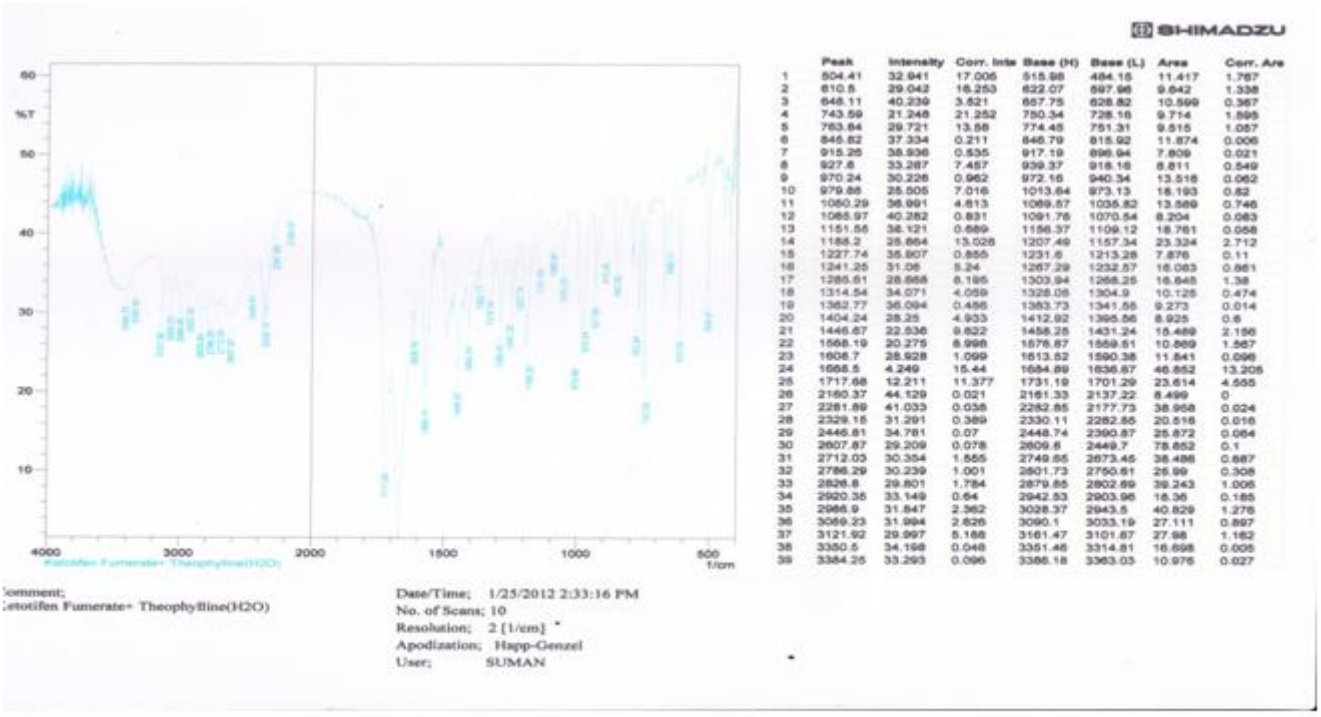
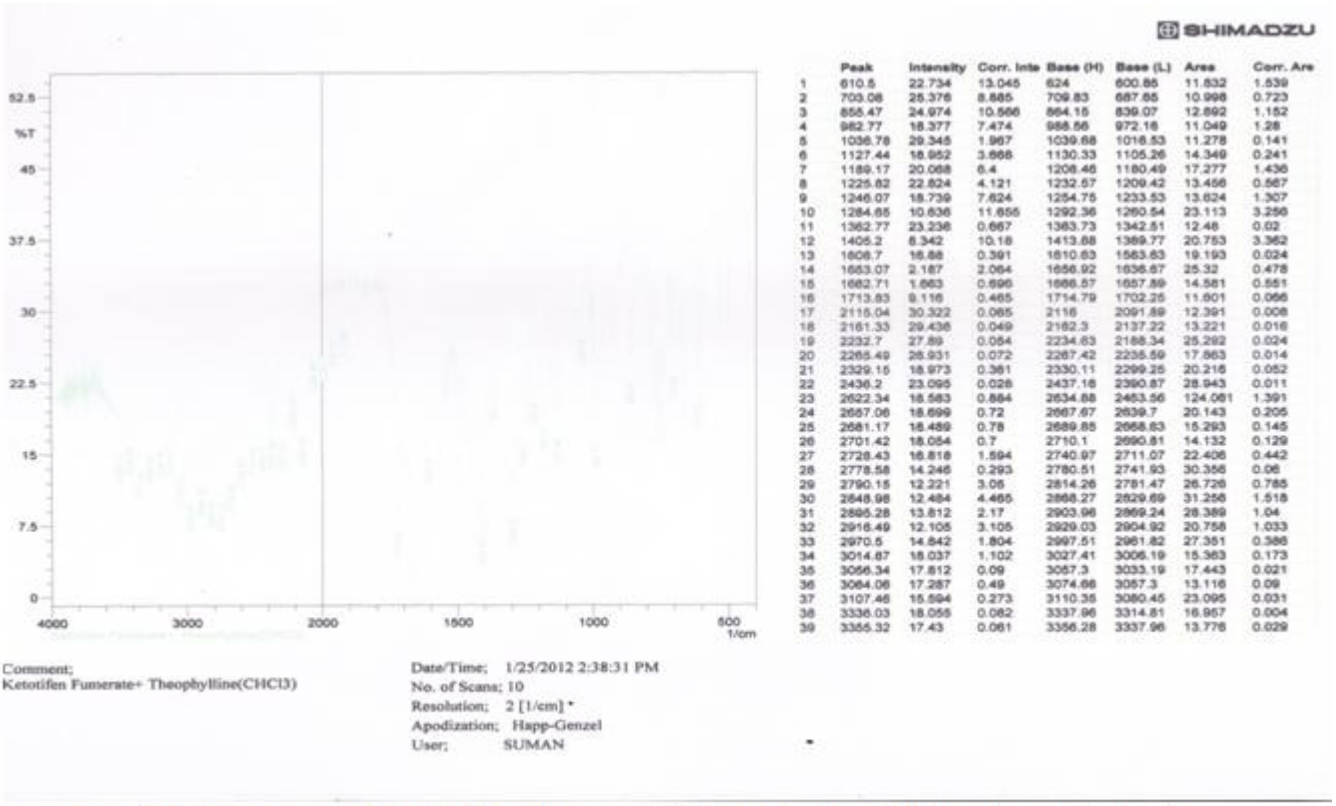


Fig. 3: IR Spectrum of Theophylline



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	504.41	32.941	17.005	515.98	484.15	11.417	1.787
2	610.5	29.042	16.253	622.07	597.96	9.842	1.338
3	648.11	40.239	3.521	657.75	626.82	10.599	0.367
4	743.59	21.248	21.252	750.34	728.16	9.714	1.595
5	763.84	29.721	13.88	774.48	751.31	9.515	1.057
6	846.82	37.334	0.211	848.79	815.92	11.874	0.006
7	915.28	38.936	0.535	917.19	896.94	7.809	0.021
8	927.8	33.287	7.457	939.37	918.16	8.811	0.549
9	970.24	30.226	0.962	972.16	940.34	13.516	0.062
10	979.88	25.505	7.016	1013.64	973.13	18.193	0.82
11	1060.29	36.991	4.613	1069.57	1035.82	13.589	0.746
12	1085.97	40.282	0.831	1091.76	1070.54	8.204	0.083
13	1151.56	38.121	0.689	1156.37	1109.12	18.761	0.058
14	1188.2	25.864	13.028	1207.49	1157.34	23.324	2.712
15	1227.74	35.907	0.855	1231.6	1213.28	7.876	0.11
16	1241.25	31.06	5.24	1267.29	1232.57	16.083	0.861
17	1285.61	28.668	8.195	1303.94	1268.25	16.845	1.38
18	1314.54	34.071	4.059	1328.08	1304.9	10.125	0.474
19	1362.77	36.094	0.486	1383.73	1341.56	9.273	0.014
20	1404.24	28.25	4.933	1412.92	1395.56	8.925	0.6
21	1446.67	22.536	9.822	1458.25	1431.24	18.469	2.156
22	1568.19	20.275	8.998	1576.87	1559.51	10.869	1.567
23	1608.7	28.928	1.099	1613.52	1590.38	11.841	0.096
24	1668.5	4.249	15.44	1684.89	1636.67	46.852	13.205
25	1717.68	12.211	11.377	1731.19	1701.29	23.614	4.555
26	2160.37	44.129	0.021	2161.33	2137.22	8.499	0
27	2281.89	41.033	0.035	2282.85	2177.73	38.958	0.024
28	2329.15	31.291	0.389	2330.11	2282.85	20.516	0.016
29	2446.81	34.781	0.07	2448.74	2390.87	25.872	0.064
30	2607.87	29.209	0.078	2609.8	2449.7	78.852	0.1
31	2712.03	30.354	1.555	2749.65	2673.45	38.486	0.687
32	2786.29	30.239	1.001	2801.73	2750.81	25.99	0.308
33	2826.8	29.801	1.784	2879.85	2802.89	39.243	1.006
34	2920.36	33.149	0.64	2942.53	2903.96	18.36	0.185
35	2966.9	31.847	2.362	3028.37	2943.5	40.829	1.276
36	3059.23	31.994	2.826	3090.1	3033.19	27.111	0.897
37	3121.92	29.997	5.188	3161.47	3101.67	27.98	1.162
38	3350.5	34.168	0.048	3351.46	3314.81	16.698	0.005
39	3384.25	33.293	0.096	3386.18	3363.03	10.976	0.027

Fig. 4: IR Spectrum of Ketotifen Fumarate + Theophylline (Aqueous Extract)



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	610.5	22.734	13.045	624	600.86	11.832	1.539
2	703.08	25.376	8.885	709.83	687.65	10.998	0.723
3	855.47	24.974	10.566	864.15	839.07	12.892	1.152
4	982.77	18.377	7.474	988.56	972.16	11.049	1.28
5	1036.78	29.345	1.967	1039.68	1016.53	11.278	0.141
6	1127.44	18.952	3.868	1130.33	1105.26	14.349	0.241
7	1189.17	20.068	6.4	1208.46	1180.49	17.277	1.436
8	1225.82	22.824	4.121	1232.57	1209.42	13.456	0.567
9	1246.07	18.739	7.624	1254.75	1233.53	13.624	1.307
10	1284.65	10.636	11.655	1292.36	1260.54	23.113	3.256
11	1362.77	23.236	0.667	1383.73	1342.51	12.48	0.02
12	1405.2	8.342	10.18	1413.88	1389.77	20.753	3.362
13	1606.7	16.88	0.391	1610.63	1583.63	19.193	0.024
14	1653.07	2.187	2.064	1656.92	1636.67	25.32	0.478
15	1662.71	1.663	0.696	1666.57	1657.89	14.581	0.551
16	1713.83	9.116	0.465	1714.79	1702.25	11.601	0.066
17	2115.04	30.322	0.085	2116	2091.89	12.391	0.008
18	2161.33	29.436	0.049	2162.3	2137.22	13.221	0.016
19	2232.7	27.89	0.054	2234.63	2188.34	25.292	0.024
20	2265.49	26.931	0.072	2267.42	2235.59	17.863	0.014
21	2329.15	18.973	0.361	2330.11	2299.25	20.216	0.052
22	2436.2	23.095	0.028	2437.16	2390.87	28.943	0.011
23	2622.34	18.583	0.884	2634.88	2453.56	124.081	1.391
24	2667.06	18.699	0.72	2667.67	2639.7	20.143	0.205
25	2681.17	18.489	0.78	2689.85	2668.63	15.293	0.145
26	2701.42	18.054	0.7	2710.1	2690.81	14.132	0.129
27	2728.43	16.818	1.594	2740.97	2711.07	22.406	0.442
28	2778.58	14.246	0.293	2780.51	2741.93	30.356	0.06
29	2790.15	12.221	3.06	2814.26	2781.47	26.726	0.785
30	2848.98	12.484	4.465	2868.27	2829.69	31.256	1.518
31	2895.28	13.812	2.17	2903.96	2869.24	28.389	1.04
32	2916.49	12.105	3.105	2929.03	2904.92	20.758	1.033
33	2970.5	14.842	1.804	2997.51	2961.82	27.351	0.386
34	3014.87	18.037	1.102	3027.41	3006.19	15.383	0.173
35	3056.34	17.612	0.09	3057.3	3033.19	17.443	0.021
36	3064.06	17.287	0.49	3074.66	3057.3	13.116	0.09
37	3107.46	15.594	0.273	3110.35	3080.45	23.095	0.031
38	3336.03	18.055	0.082	3337.96	3314.81	16.957	0.004
39	3355.32	17.43	0.061	3356.28	3337.96	13.776	0.029

Fig. 5: IR Spectrum of Ketotifen Fumarate + Theophylline (Chloroform Extract)

Table 2: Interacted peaks for Ketotifen Fumarate and Theophylline

Aqueous extract Wave No. (cm^{-1})	Chloroform extract Wave No. (cm^{-1})
763.84	703.08
1595.2	2115.04
2281.89	2161.33
2625.23	2232.7
2729.39	2657.06
3384.25	2701.42

Discussion

There are some peaks observed in the spectrum of combination of the two drugs Ketotifen Fumarate and Theophylline in both aqueous and chloroformic extracts as compared to that of pure forms. From these extra peaks we confirm that there is an interaction between Ketotifen Fumarate and Theophylline in combination form. By observing the graph obtained by plotting invivo results, we can see that the absorbances of Ketotifen fumarate is decreased when administered in combination with Theophylline and vice-versa. We know that the value of absorbance is directly proportional to the drug concentration from beer and lambart's law. Since the absorbance is decreased, the plasma drug concentration also decreases which reflects there might be drug interaction due to which the plasma drug concentration is decreased. Hence we can conclude that there might be drug interaction between Ketotifen fumarate and Theophylline.

There are many extra peaks observed in the IR spectra of interacted product of ketotifen fumarate with Theophylline in the mixture ratio of 1:1 either in aqueous or chloroform extracts, in compared to that of their pure form. The presence of the extra peaks confirmed that there is a interaction between Ketotifen fumarate and Theophylline.

Conclusion

From the in-vivo and IR study it is confirmed that there are interaction between Ketotifen fumarate and Theophylline when administered concurrently. Therefore cautions must be taken when these combination drugs are administered to minimize the risk of drug interaction and get maximum therapeutic efficacy of the individual drugs to cure the illness of the patient in its rational use. Further studies can be carried out to determine whether these drug interactions are beneficial or harmful.

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