



**IN - VIVO STUDY ON INTERACTION OF KETOTIFEN FUMERATE WITH DI -
CLOFENAC - NA ALONG WITH THEIR IR STUDIES**

**Md. Khalequeuzzaman¹, Md. Mominur Rahman², Mst. Rozina Parul*¹, Mohammed Aktar Sayeed²,
Md. Nazmul Hasan³**

¹Department of Pharmacy, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka - 1344.

²Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

³Department of Pharmacy, Jahangirnagar University, Savar, Dhaka – 1342.

***Corresponding Author: Rozina Parul**

Department of Pharmacy, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka - 1344.

Article Received on 26/04/2016

Article Revised on 16/05/2016

Article Accepted on 06/06/2016

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 Di-Clofenac Na along with their IR studies. In-vivo study showed decreased in the plasma concentration of free Ketotifen Fumarate when given concurrently with Di-Clofenac Na which may be due to the drug interaction. IR study confirmed that there is an interaction between Ketotifen Fumarate and Diclofenac Na with the presence of extra peaks in their combination (aqueous & chloroform extract) forms as compared to that of their pure forms. So, we can say that if both ketotifen fumerate with Di-Clofenac administer concurrently, mild complex can be formed after reaction which can ultimately reduce the pharmacological activities of both combinations of drugs.

KEYWORDS: *Kitotifen fumerate, Di-Clofenac Na, pharmacological activities & drug interactions.*

1. 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 nowadays 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 H₁ receptors, inhibits calcium uptake, blocks passive cutaneous anaphylactic reaction, reverses isoprenalineinduced 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]

Diclofenac are anti-inflammatory painkillers sometimes called non-steroidal anti-inflammatory drugs (NSAIDs), or just 'anti-inflammatories'. Di-Clofenac is used to treat painful conditions such as: arthritis, Rheumatic arthritis, Osteoarthritis, Acute musculoskeletal injury, sprains and strains, gout, migraine, Dysmenorrhoea, Dental pain, and pain after surgical operations. It ceases pain and reduces inflammation.

The research result showed that concurrent administration of Ketotifen Fumerate and potassium nitrate with diltiazem or with nifedipine did not make a significant change on the antihypertensive activity of diltiazem or nifedipine. Further considerations and monitoring are to be practiced during concurrent therapy of diltiazem and nifedipine to avoid untoward pharmacological and therapeutic actions related to drug interactions but coadministration of Ketotifen Fumerate and potassium nitrate with either diltiazem or nifedipine might be regarded as safe and effective.^[7] The results showed that the *in vitro* availability of NSAIDs and metformin owing to interaction was depressed through the formation of charge transfer complexes which was found to be associated with inter- and intra-molecular rearrangement of the electronic cloud of the interacting drugs. Hence it is envisaged that concurrent administration of metformin and NSAIDs could alter the bio-availability and impair the clinical efficacy of both drugs. Effect of pH is also studied on these drug-drug interactions.^[8] The study was to evaluate the effect of betamethasone and Di-Clofenac sodium on serum and tissue concentration of amoxicillin in rats. Considering single doses, betamethasone did not interfere with amoxicillin levels but Di-Clofenac sodium reduced both tissue and serum levels of amoxicillin in rats.^[9]

2. MATERIALS AND METHODS

2.1 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.

2.2 Drugs and chemicals

Ketotifen Fumerate and Di-Clofenac Na were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh as a token gift and were used without further purification. 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 analytical grade.

2.3 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.

2.4 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
Di-Clofenac Na	276nm

2.5 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.

2.6 Preparation of drugs solutions

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

Doses: Ketotifen Fumarate: 0.1mg/kg

Di-Clofenac Na: 10mg/kg

2.7 METHODOLOGY

2.7.1 For Ketotifen Fumarate + Diclofenac Na

48 fresh mice were separated for the experiment which was 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 0 min. 3 mice were sacrificed after 30 mins, blood sample were collected and their absorbances were taken @ 300 nm. Similarly every 3 mice were sacrificed after 60 mins, 120 mins & 180 mins 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 0min. 3 mice were sacrificed after 30 mins, blood sample were collected and their absorbances were taken @ 300 nm. Similarly every 3 mice were sacrificed after 60 mins, 120 mins & 180 mins and the same procedure were followed as above.

Group III

They were administered Diclofenac Na 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 @ 276 nm. Similarly every 3 mice were sacrificed after 60 mins, 120 mins & 180 mins and the same procedure were followed as above.

Group IV

They were administered mixture of ketotifen fumarate + Diclofenac Na solution (1:1) 0.4 ml 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 @ 300 nm and 276 nm. Similarly every 3 mice were sacrificed after 60 mins, 120 mins & 180 mins and the same procedure were followed as above.

- 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.

2.7.2 For Infrared Analysis

The interaction between the drug were analysed by IR spectrum analyses

For analysis of drug interaction between Ketotifen fumarate & Diclofenac Na

- 100mg of pure powder of Ketotifen fumarate was dissolved in 10ml of distilled water in a 50ml beaker; similarly 100mg of pure powder of Diclofenac Na was dissolved in another beaker.

3. RESULT AND DISCUSSION

3.1 In-vivo study Ketotifen Fumarate and Diclofenac Na

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

Absorbances at 300nm	30min		60min		120min		180min	
	0.085	0.064±0.134	0.078	0.066±0.006	0.067	0.077±0.006	0.889	0.362±0.263
	0.068		0.056		0.075		0.096	
	0.039		0.065		0.089		0.102	

Group II (Ketotifen Fumarate)

Absorbances at 300nm	30min		60min		120min		180min	
	1.107	1.143±0.027	0.791	0.852±0.031	0.685	0.719±0.018	0.756	0.788±0.018
	1.127		0.872		0.729		0.789	
	1.196		0.894		0.744		0.819	

Group III (Diclofenac Na)

Absorbances at 272nm	30min		60min		120min		180min	
	2.792	2.826±0.019	2.867	2.830±0.021	2.516	2.540±0.018	2.372	2.333±0.029
	2.829		2.829		2.527		2.352	
	2.858		2.796		2.577		2.275	

Group IV (Ketotifen Fumarate + Diclofenac Na)

Absorbances at 300nm	30min		60min		120min		180min	
	0.866	0.914±0.026	0.194	0.145±0.026	0.116	0.082±0.041	0.256	0.36±0.015
	0.923		0.105		0.001		0.207	
	0.955		0.137		0.129		0.246	

Absorbances at 272nm	30min		60min		120min		180min	
	1.907	1.907±0.016	1.773	1.715±0.033	1.542	1.532±0.027	1.478	1.454±0.003
	1.875		1.715		1.483		1.389	
	1.931		1.658		1.572		1.496	

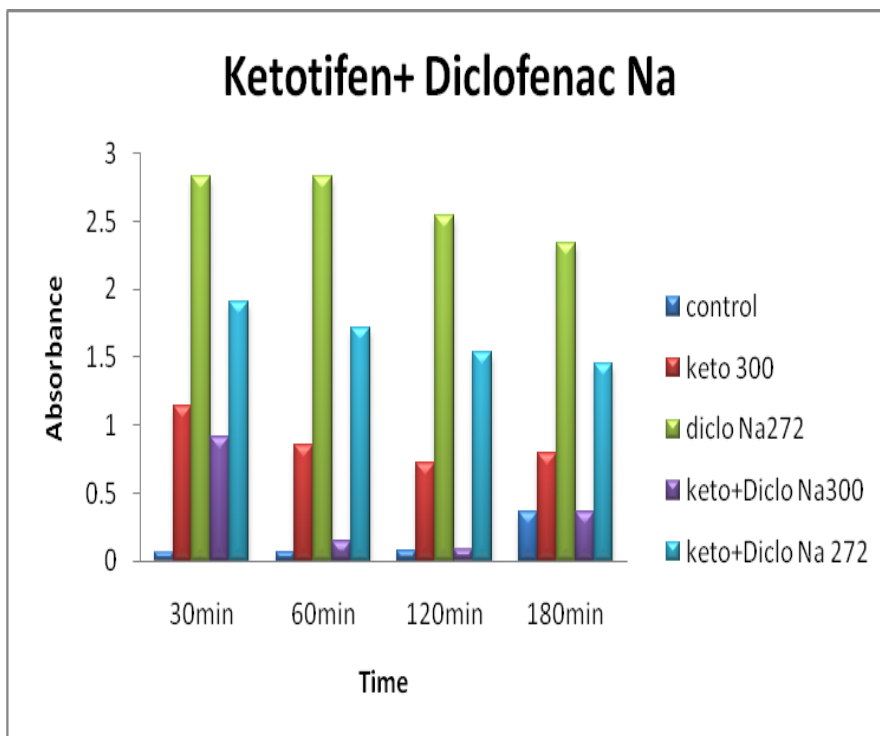


Fig. 1: Graph for Ketotifen fumarate+ Diclofenac Na interaction.

3.2 Infrared Study

IR Spectrum of Ketotifen Fumarate

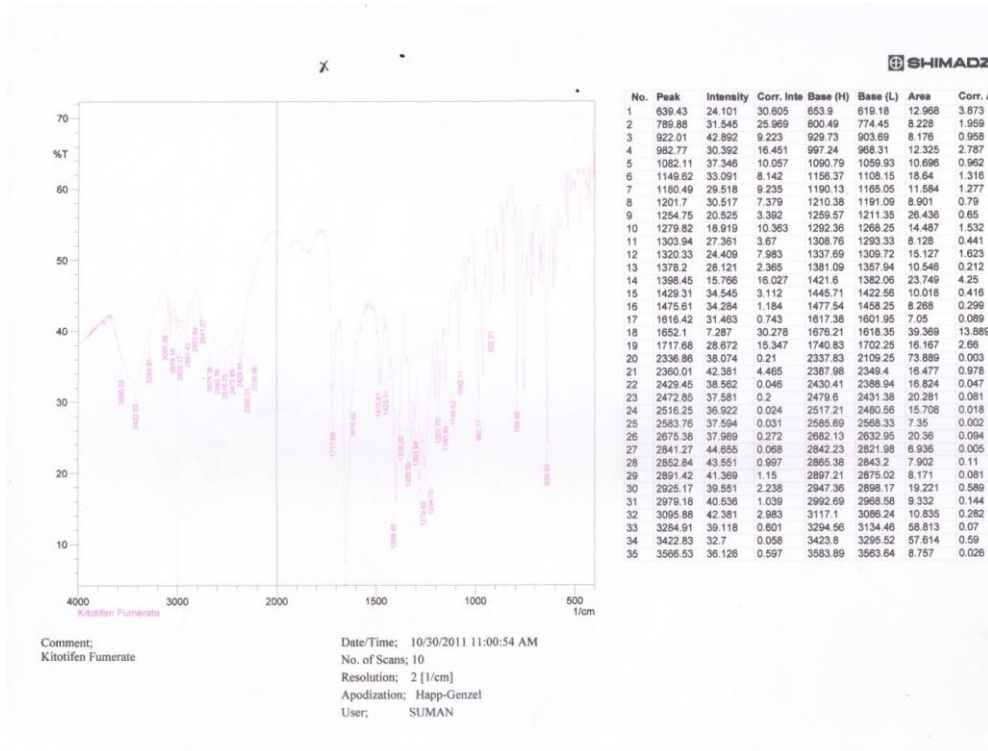


Fig. 2: IR Spectrum of Ketotifen Fumarate

IR Spectrum of Diclofenac Sodium

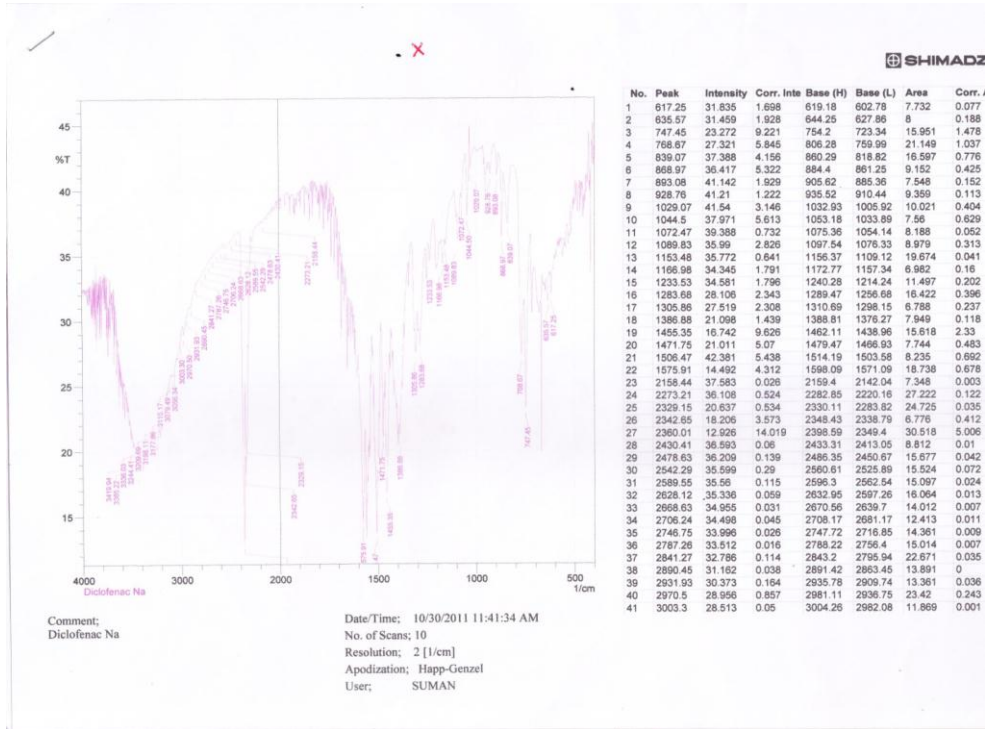


Fig. 3: IR Spectrum of Diclofenac Sodium

IR Spectrum of Ketotifen Fumarate + Diclofenac Na (Aqueous Extract)

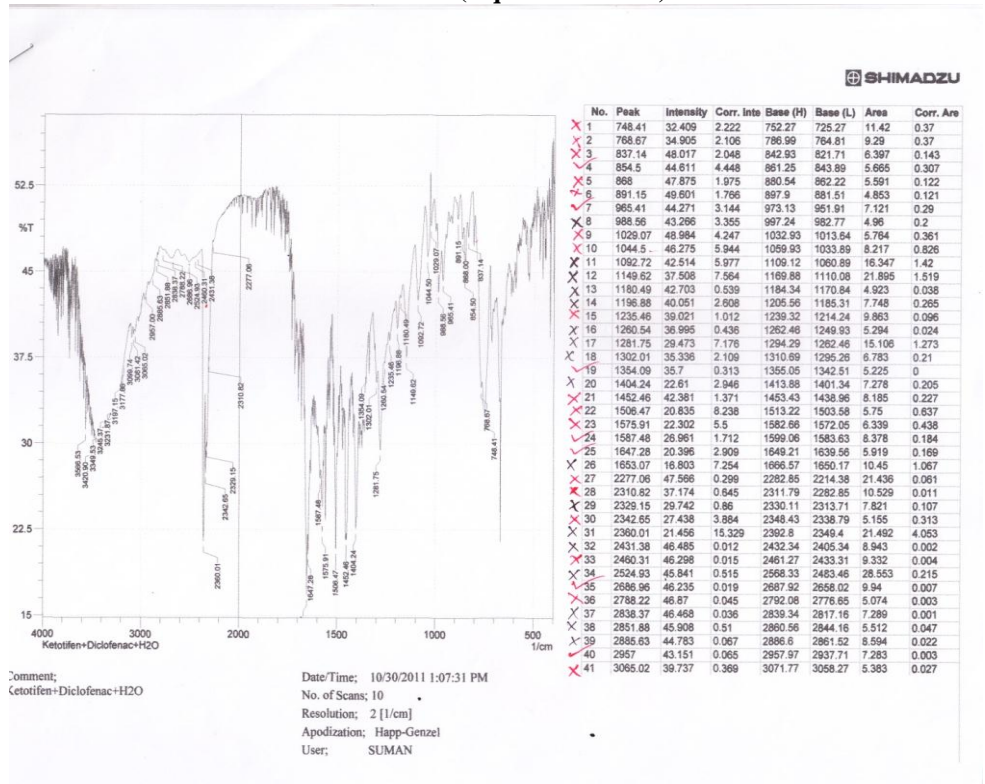
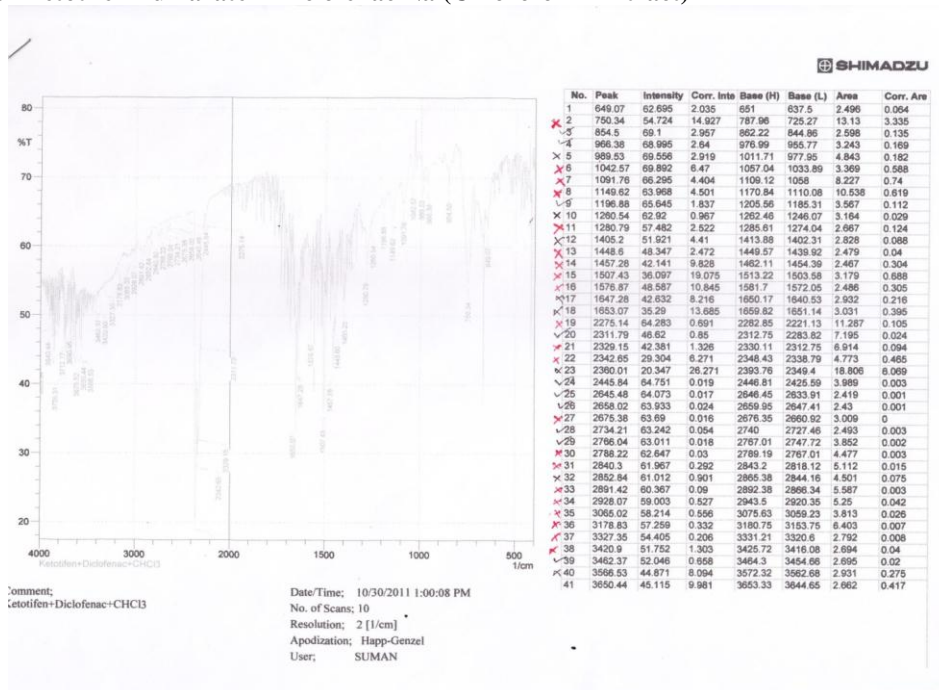


Fig. 3: IR Spectrum of Ketotifen Fumarate + Diclofenac Na (Aqueous Extract)

IR Spectrum of Ketotifen Fumarate + Diclofenac Na (Chloroform Extract)



3.3 Interacted peaks for Ketotifen Fumarate and Diclofenac Na

Table 2: Interacted peaks for Ketotifen Fumarate and Diclofenac Na

Aqueous extract Wave No. (cm ⁻¹)	Chloroform extract Wave No. (cm ⁻¹)
854.5	854.5
965.41	966.38
1354.09	1196.88
1587.48	2311.79
1647.28	2445.84
2957	3462.37

3.4 DISCUSSION

There are some peaks observed in the spectrum of combination of the two drugs Ketotifen Fumarate and Diclofenac Na 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 Diclofenac Na in combination form. By observing the graph obtained by plotting in vivo results, we can see that the absorbances of Ketotifen Fumarate is decreased when administered in combination with Diclofenac Na and vice-versa. We know that the value of absorbance is directly proportional to the drug concentration from Beer and Lambert'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 Diclofenac Na. There are many extra peaks observed in the IR spectra of interacted product of Ketotifen Fumarate with Diclofenac Na in the mixture ratio of 1:1 either in aqueous or chloroform extracts, in compared to that of their pure form. We think the appearance of extra

peaks in their combined form may be due to interaction between Ketotifen Fumarate and Diclofenac Na.

4. CONCLUSION

From the in vivo and IR study it is confirmed that there are interaction between Ketotifen Fumarate and Diclofenac Na when administered concurrently. Therefore cautions must be taken when this combination drug is 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.

5. REFERENCES

- Martin U, Romer E. Ketotifen: a histamine release inhibitor. *Monogr Allergy*, 1977; 12: 145-149.
- Craps L, Greenwood C, Radielovic P. Clinical investigation of agents with prophylactic anti-allergic effects in bronchial asthma. *Clinical Allergy*, 1978; 8: 373-382.
- Hoshino M, Nakamura Y, Sim JJ, Tomioka H A comparative study of the effects of ketotifen, disodium cromoglycate and beclomethasone dipropionate on bronchia mucosa and asthma

- symptoms in patients with atopic asthma. *Respiratory Medicine*, 1998; 92: 942- 950.
4. Tinkelman D G, Moss B A, Bukantz S C, Sheffer A L, Dobken J H, Chodosh S et al. A multicenter trial of the prophylactic effect of ketotifen, amoxicillin, and placebo in atopic asthma. *J. Allergy Clin. Immunol.*, 1985; 76: 487-497.
 5. Clarke C W, May C S. A comparison of the efficacy of ketotifen (HC 20-511) with sodium cromoglycate (SCG) in skin test positive asthma. *British Journal of Clinical Pharmacology*, 1980; 10: 473-476.
 6. Church K M, Gradidge C F. Inhibition of histamine release from human lung in vitro by antihistamines and related drugs. *British Journal of Pharmacology*, 1980; 69: 663-667.
 7. Md Shah Amran, Md Rahatuzzamn and Md Amjad Hossain “The synergism of diltiazem and nifedipine in their antihypertensive functions in the animal model and the case of coadministration of ketotifen fumerate and potassium nitrate with them” *Journal of bio-science.*, 2006; 14: 61-67, ISSN 1023-8654.
 8. Saeed Arayne Najma Sultana M, Hashim Zuberi and Urooj Haroon M. “In vitro studies of interaction between metformin and nsaid (non steroidal anti-inflammatory drugs) using spectrophotometry and rp-high performance liquid chromatography” *Journal of the Chilean Chemical Society versión On-line* ISSN 0717-9707.
 9. Thales Rocha Mattos filho, Marcelo de Souza Junqueira, Francisco Carlos Groppo, Rogério Heládio Lopes MOTTA, Fábio Ferreira PERAZZO “Effect of betamethasone and diclofenac sodium on serum and tissue concentration of amoxicillin. In vivo study in rats” *Journal of Applied Oral Science.*, 2006; 14(5): 319-23.