VOLUME-6 | ISSUE-1 | JANUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

To identify in vitro effective combination of Isoniazid and Rifampicinwith Isoxyl against Multi Drug Resistant and susceptible strains of *Mycobacterium tuberculosis* and Mycobacteria Other Than Tuberculosis strains by checkerboard titration.

Pathology

KEYWORDS: Mycobacterium tuberculosis, Isoniazide, Rifapmicin, Isoxyl, Mycobacteria Other Than Tuberculosis (MOTT) strains

Shashikant Vaidya	Clinical Pathology department, Haffkine Institute, Mumbai, India
Shreyasi Muley	Haffkine Bio-pharmaceutical Corporation Ltd. Mumbai, India
Geeta Koppikar	Breach Candy hospital trust, Mumbai, India
M.G. Kulkarni	T.N. Medical College and B.Y.L. Nair Charitable Hospital, Mumbai, India
Abhay Chowdhary	Grant Medical College and Sir J.J. Group of Hospitals, Mumbai, India

ABSTRACT

When two antibacterial agents act simultaneously upon a uniform microbial population, the result may be addition, synergism, antagonism or indifference.Interest in the use of combination of antibiotics was started many years ago, almost as soon as two antibiotics were available. This study is carried out to identify in vitro effective combination of Isoniazide (INH) and Rifapmicin (RF) with Isoxyl (ISO) against MDR and susceptible strains of Mycobacterium tuberculosis and Mycobacteria Other Than Tuberculosis (MOTT) strains by checkerboard titration using Microplate Alamar Blue Assay(MABA) in terms of Minimum Inhibitory Concentration(MIC) values.Total 20 MDR strains , 10 susceptible strains of M. tuberculosis and Four MOTT strains were included in the study.When INH & ISO and RF & ISO were used in combination against resistant and susceptible strains of M. tuberculosis and MOTT strains, enhancement of inhibitory activity of INH and RF was observed. Results are depicted by Fractional Inhibitory concentration Index (FIC).When FIC index is less than or equal to 0.5, combination is synergistic .When FIC index is more than or equal to 2.0, combination is antagonistic. When FIC index is equal to 1.0, combination is additive. The MICs of INH dropped more than RF along with ISO against resistant and susceptible strains of M. tuberculosis and MOTT strains. There was an in vitro synergistic interaction between ISO & INH and ISO & RF to M.tuberculosis and MOTT strains.ISO and INH combination was more synergistic than ISO and RF combination.It appears that ISO had biological effect in the prevention of emergence of resistant strains, to standard drugs and as a corollary, must be efficacious as an antituberculosis drug in combination therapy.

INTRODUCTION:

Interest in the use of combination of antibiotics was started many years ago, almost as soon as two antibiotics were available. This corresponded to a belief among physicians that antimicrobial agents are widely effective and fairly harmless and in their search for security in treatment they prescribe antibiotic combinations because of a general feeling that if one antibiotic is good, two should be better and three should cure almost everybody of almost every ailment.[1]

Emotions such as these probably contribute to the large-scale abuse of antimicrobial drugs and in turn for the high incidence of side effects to drugs among patients and for the rise of antimicrobial resistance among microorganisms. Nevertheless there are clear-cut situations where the simultaneous use of two or more antimicrobial drugs is essential for the survival of the patient or the eradication of an infection.

Decreased emergence of resistant microbes has been best shown in the chemotherapy of Tuberculosis (TB) where the frequency of resistant strains appearing during therapy has been reduced by the simultaneous use of multiple drugs. [2].The administration of Gentamycin in conjuction with Carbenicillin for infections due to *Pseudomonasaeruginosa* has been proposed specifically to decrease the selection of Carbenicillin-resistant mutants.[3].

The use of triple-sulphas avoids urinary blockage with crystals of sulphadia-zine. The explanation for this effect is that the solubility of each component (Sulphadiazine, Sulphamerazine, and Sulphamethazine) in urine is independent of the others, although their antibacterial activity is cumulative. [4].This is lessening the dose related toxicity of treatment:

In mixed infections, it is possible that two or more drugs, each acting on a separate portion of a complex microbial flora may be more effective than one. For instance, the mixture of aerobic and anaerobic bowel flora causing peritonitis after perforation of an intestinal viscus. [1]At times, the simultaneous use of two drugs achieves an effect not obtainable by either of the drugs alone. One drug may specifically enhance the antibacterial activity of the second drug against a specific microorganism. [1]Eg. Carbenicillin plus Gentamicin or Tobramycin for *P. aeruginosa*. [5].Thus there is increased bacterial killing by drug synergism.

Combination therapy is often prescribed by physicians in serious infections, where there is no clue as to the nature and susceptibilities of the infecting agent or there is a delay in bacteriological investigation.[6].

When two antibacterial agents act simultaneously upon a uniform microbial population, the result may be addition, synergism, antagonism or indifference. [7]

Rationale for drug combination in chemotherapy of Mycobacterial infections are as follows.

The use of antimicrobial in combination has been known since the discovery of the first antibiotics. A combination of Penicillin with Streptomycin was effective in the therapy of enterococcal endocarditis, whereas Penicillin alone was not effective.[1] At the very beginning of the era of antibiotic, it was also observed that combinations of antimicrobial agents were not always more effective than single drugs. Some combinations were even found to be harmful or antagonistic. Combinations of antimicrobial agents are most often used for the following reasons: [1][8]

To minimize the probability of emergence of drug resistance, to increase the activity (particularly the bactericidal activity) of the agents which have bactericidal effect, to reduce a potentially toxic effect by employing lower dosages of each drug, to provide broad coverage of infections caused by unidentified organisms and for treatment of polymicrobial infections.

The rationale for combination chemotherapy of Mycobacterial infections varies, depending on the causative agent. Use of various combined antituberculosis drugs is one of the most important

principles of the chemotherapy of TB. As discussed, the main reason for introducing this therapy was the prevention of drug resistance. Later, the use of multi-drug regimens was found to be instrumental in improving the rate of success of therapy of patients in geographical areas where there was a high incidence of initial drug resistance. Current use of drug combinations in the therapy of TB is aimed at designing the most efficient short-course regimens.This study is carried out to identify in vitro effective combination of Isoniazide (INH) and Rifapmicin (RF) with Isoxyl (ISO) against MDR and susceptible strains of *Mycobacterium tuberculosis* and Mycobacteria Other Than Tuberculosis (MOTT) strains by checkerboard titration using Microplate Alamar Blue Assay(MABA) in terms of Minimum Inhibitory Concentration(MIC) values.[9] [10].Standard strain of *M. tuberculosis* H37Rv was also tested against all these drugs namely Isoniazide (INH) and Rifapmicin (RF) with Isoxyl (ISO)

MATERIAL AND METHODS:

Total 20 MDR strains and 10 susceptible strains of *M. tuberculosis* were collected from Department of Microbiology of P.D. Hinduja Hospital and Medical Research Centre which were tested for drug susceptibility testing by Bactec460TB system.[11].Four MOTT strains were also included in the study. All clinical isolates were defined as MOTT according to their growth rates, pigmentation properties of colonies, susceptibility to para-nitrobenzoic acid, semiquantitative catalase test, nitrate reduction test and niacin accumulation tests –().Standard strain of *M. tuberculosis* H37Rv was also tested against all these drugs. Study was carried out in Department Of Microbiology, B.Y. L. Nair Charitable Hospital and T.N. Medical College Mumbai.

Serial two fold dilutions of individual drug was prepared in Sterile Dubos broth with glucose and albumin supplements (HiMedia Laboratories)after dissolving it in suitable diluents. Drug concentrations (mcg/ml) used. Any six concentrations of the drugs were used as per the MIC of that test strain.

i.) ISO (Cayman Chemicals): 0.035, 0.07, 0.15, 0.3, 0.6, 1.2, 2.5, 5, 10 and 20.

ii.) INH (Lupin Pharmaceuticals): 0.006, 0.012, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8,1.6 and 3.2

iii.) RF (Lupin Pharmaceuticals): 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16 and 32.

Culture suspension was prepared as mentioned in the procedure of MABA.[9]

Sterile water for injection in 200-microlitre quantities was added in H1 to H12 wells of 1st microtitre plate used for MIC determination. (Refer Figure No.1). Sterile water for injection in 200 microlitre quantities was added in H1 to H12 wells and A1 to A12 wells of 2nd microtite plate used for checkerboard titration study.(Refer Figure No.1). 1 to 8 wells of Row No. 1 to Row 6 were used for one titration, while 1 to 8 wells of Row No. 7 to 12 were used for second titration.(Refer Figure No.2). Appropriate amount of drug solutions were added in a checkerboard manner as shown in figure.

1st microtitre plate was used for determining MICs of INH, RF & ISO of two test strains in duplicate. (Refer Figure No.1). Addition of drug solutions were carried out by using multichannel automated pipette in a similar manner mentioned in MABA assay.In each well 100 microlitre of test culture was added. The plates were sealed with parafilm and incubated at 37^{0}C for 8 days. 50 microlitre of freshly prepared 1:1 mixture of 10-x Alamar blue reagent and 10% Tween 80 solution was added to each well.The plates were reincubated at 37^{0}C for 24 hrs and the colour of the all the wells were recorded. A blue colour in the well was interpreted as no growth and pink colour was scored as growth.Before adding reagent mixture on 10th day to all the wells, the growth was confirmed in the positive control.

Arrangements of checkerboard titrations are as shown in the Figure.

Figure N0. 1: Plate 1

	A	B										
	A1		B1		C1		A2		B2		C2	
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0
G	M	+	+	+	+	+	M	+	+	+	+	+
H	0	0	0	0	0	0	0	0	0	0	0	0

Keys:

0: Wells containing 200 microlitre of sterile water for injection (H1 to H_{12})

+: Positive control (G2 to G_6: for 1st strain G_8 to G_{12}: for 2nd strain)

M: media control (G1 & G_7)

A: MIC determination of 1st strain, B: MIC determination of 2nd strain

A_1: Drug Concentrations of ISO in ascending manner for 1st strain.
B_1: Drug Concentrations of INH in ascending manner for 1st strain.
C_1: Drug Concentrations of RF in ascending manner for 1st strain.

A_2: Drug Concentrations of ISO in ascending manner for 2nd strain.
B_2: Drug Concentrations of INH in ascending manner for 2nd strain.
C_2: Drug Concentrations of RF in ascending manner for 2nd strain.

Figure N0. 2: Plate 2

	A	B										
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0	0	0	0

Keys:

0: wells containing 200 microlitre of sterile WFI (A1 to A 12 and H1 to H12)

A: ISO + INH combination study (Checkerboard titration)

B: ISO + RF combination study (Checkerboard titration)

Figure N0. 3:Checkerboard titration

Drug B (mcg/ml)	400	32:1	16:1	8:1	4:1	2:1	1:1
	200	16:1	8:1	4:1	2:1	1:1	1:2
	100	8:1	4:1	2:1	1:1	1:2	1:4
	50	4:1	2:1	1:1	1:2	1:4	1:8
	25	2:1	1:1	1:2	1:4	1:8	1:16
	12.5	1:1	1:2	1:4	1:8	1:16	1:32
	0	12.5	25	50	100	200	400
	Drug A (mcg/ml)						

Results are depicted by Fractional Inhibitory concentration Index (FIC).

VOLUME-6 | ISSUE-1 | JANUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

When FIC index is less than or equal to 0.5, combination is synergistic
When FIC index is more than or equal to 2.0, combination is antagonistic
When FIC index is equal to 1.0, combination is additive

The FIC index is calculated by using the following formula:
FIC index= FIC of drug A + FIC of drug B

While,
FIC of drug A= MIC of drug A in combination with B / MIC of drug A

FIC of drug B= MIC of drug B in combination with A / MIC of drug B

RESULTS:

Table No 1: Checkerboard titrations of INH + ISO

Sr. No.	Strain	MIC of INH	MIC of ISO	FIC				
		Alone	Combination	Alone	Combination	INH	ISO	Index
1	MDR20	0.1	0.5	2.5	1.25	0.5	0.5	1
2	MDR 21	0.2	0.1	0.6	0.3	0.5	0.5	1
3	MDR 22	0.2	0.1	0.3	0.15	0.5	0.5	1
4	MDR 23	0.1	0.05	1.2	0.6	0.5	0.5	1
5	MDR 24	0.2	0.1	2.5	1.25	0.5	0.5	1
6	MDR 25	0.4	0.2	1.2	0.6	0.5	0.5	1
7	MDR 26	0.8	0.4	1.2	0.6	0.5	0.5	1
8	MDR 27	0.4	0.2	1.2	0.6	0.5	0.5	1
9	MDR 28	0.2	0.1	1.2	0.6	0.5	0.5	1
10	MDR 29	0.4	0.2	1.2	0.6	0.5	0.5	1
11	MDR 30	0.4	0.2	1.2	0.6	0.5	0.5	1
12	MDR 31	0.4	0.2	1.2	0.6	0.5	0.5	1
13	MDR 32	0.4	0.2	2.5	1.2	0.5	0.5	1
14	MDR 33	0.2	0.05	0.6	0.125	0.25	0.125	0.375
15	MDR 34	0.4	0.2	1.2	0.6	0.5	0.5	1
16	MDR 35	0.4	0.1	1.2	0.3	0.25	0.25	0.5
17	MDR 36	0.4	0.1	2.5	0.6	0.5	0.5	0.5
18	MDR	0.8	0.4	2.5	1.2	0.5	0.5	1
19	MDR 38	0.8	0.4	1.2	0.6	0.5	0.5	1
20	MDR 39	0.8	0.4	1.2	0.6	0.5	0.5	1
21	S1	0.05	0.006	2.5	0.3	0.125	0.125	0.25
22	S2	0.05	0.025	0.3	0.15	0.5	0.5	1
23	S3	0.05	0.012	0.15	0.035	0.25	0.25	0.5
24	S4	0.012	0.006	0.6	0.3	0.5	0.5	1
25	S5	0.012	0.006	1.2	0.6	0.5	0.5	1
26	S6	0.012	0.006	0.6	0.3	0.5	0.5	1
27	S7	0.025	0.006	0.3	0.07	0.25	0.25	0.5
28	S8	0.012	0.006	0.6	0.3	0.5	0.5	1
29	S9	0.012	0.006	0.3	0.15	0.5	0.5	1
30	S10	0.050	0.006	0.3	0.15	0.125	0.062	0.187
31	MOTT1	0.4	0.1	1.2	0.3	0.25	0.25	0.5
32	MOTT 4	0.8	0.4	2.5	1.2	0.5	0.5	1
33	MOTT 8	0.05	0.012	0.15	0.035	0.25	0.25	0.5
34	MOTT	0.2	0.05	0.6	0.125	0.25	0.125	0.375

20 MDR strains and 10 susceptible strains *M. tuberculosis* were tested by checkerboard titration to INH and ISO to determine the FIC index to determine the synergistic activity between them.

17 MDR strains of *M. tuberculosis* showed additive effect with RF and ISO combination with FIC index 1. While two MDR strains namely MDR35 and MDR36 showed synergistic effect with FIC index 0.5. While MDR33 showed synergistic effect with FIC index 0.375.

6 susceptible strains of *M. tuberculosis* showed additive effect with RF and ISO combination with FIC index 1. While strain S1 showed synergistic activity with FIC index 0.25, strains S3 and S7 showed synergistic activity with FIC index 0.5 and strain S10 showed synergistic activity with FIC index 0.187.

MOTT 1, MOTT 8 and MOTT 10 strains showed synergistic activity with FIC index 0.5, 0.5 and 0.375 respectfully. While MOTT 4strain showed additive effect

Table No 2**:** Checkerboard titrations **of RF** + ISO

Sr. No.	Strain	MIC of RF		MIC of ISO		FIC		
		Alone	Combination	Alone	Combination	RF	ISO	Index
1	MDR20	2.0	1.0	2.5	1.25	0.5	0.5	1
2	MDR 21	4.0	2.0	0.6	0.3	0.5	0.5	1
3	MDR 22	2.0	1.0	0.3	0.15	0.5	0.5	1
4	MDR	4.0	2.0	1.2	0.6	0.5	0.5	1
5	MDR 24	2.0	1.0	2.5	1.25	0.5	0.5	1
6	MDR 25	4.0	2.0	1.2	0.6	0.5	0.5	1
7	MDR 26	8.0	4.0	1.2	0.6	0.5	0.5	1
8	MDR 27	16.0	8.0	1.2	0.6	0.5	0.5	1
9	MDR 28	4.0	2.0	1.2	0.6	0.5	0.5	1
10	MDR 29	16.0	8.0	1.2	0.6	0.5	0.5	1
11	MDR 30	16.0	8.0	1.2	0.6	0.5	0.5	1

12	MDR 31	16.0	8.0	1.2	0.6	0.5	0.5	1
13	MDR 32	8.0	4.0	2.5	1.2	0.5	0.5	1
14	MDR 33	4.0	2.0	0.6	0.3	0.5	0.5	1
15	MDR 34	8.0	4.0	1.2	0.6	0.5	0.5	1
16	MDR 35	8.0	2.0	1.2	0.3	0.25	0.25	0.5
17	MDR 36	8.0	2.0	2.5	0.6	0.25	0.25	0.5
18	MDR 37	16.0	8.0	2.5	1.2	0.5	0.5	1
19	MDR 38	8.0	4.0	1.2	0.6	0.5	0.5	1
20	MDR 39	4.0	2.0	1.2	0.6	0.5	0.5	1
21	S1	0.5	0.25	2.5	1.2	0.5	0.5	1
22	S2	0.25	0.12	0.3	0.15	0.5	0.5	1
23	S3	0.25	0.06	0.15	0.035	0.25	0.25	0.50
24	S4	0.5	0.25	0.6	0.3	0.5	0.5	1
25	S5	0.25	0.12	1.2	0.6	0.5	0.5	1
27	S6	1.0	0.12	0.6	0.07	0.125	0.125	0.25
26	S7	0.5	0.25	0.3	0.15	0.5	0.5	1
28	S8	0.5	0.25	0.6	0.3	0.5	0.5	1
29	S9	0.25	0.12	0.3	0.15	0.5	0.5	1
30	S10	0.25	0.12	0.3	0.15	0.5	0.5	1
31	MOTT 1	8.0	2.0	1.2	0.3	0.25	0.25	0.5
32	MOTT 4	8.0	4.0	1.2	0.6	0.5	0.5	1
33	MOTT 8	16.0	8.0	1.2	0.6	0.5	0.5	1
34	MOTT 10	0.8	0.4	2.5	1.2	0.5	0.5	1

20 MDR strains and 10 susceptible strains *M. tuberculosis* were tested by checkerboard titration to RF and ISO to determine the FIC index to determine the synergistic activity between them.

18 MDR strains of *M. tuberculosis* showed additive effect with RF and ISO combination with FIC index 1. While two MDR strains namely MDR35 and MDR36 showed synergistic effect with FIC index 0.5.

8 susceptible strains of *M. tuberculosis* showed additive effect with RF and ISO combination with FIC index 1. While strain S3 and S6 showed synergistic activity with FIC index 0.5 and 0.25.

MOTT 1 strain showed synergistic activity with FIC index 0.5.While MOTT 4, MOTT 8 and MOTT 10 strains showed additive effect.

DISCUSSION:

Synergism can be evaluated by the checkerboard method and by determining the rate of killing of bacteria by the combination and by the individual drugs. For clinical investigations both techniques help to define optimally, the in vitro phenomena of synergism and its relation to clinical situations. [10]

The checkerboard assay was performed for a few representative clinical isolates of *M tuberculosis*. The strains used in this study were resistant to two or more antitubercular drugs as well as susceptible to antitubercular drugs. The checkerboard patterns obtained have been represented by estimating fractional inhibitory concentration index, a method of quantitating synergism suggested by Elion et al (1954). [12]

When INH & ISO and RF & ISO were used in combination against resistant and susceptible strains, enhancement of activity was observed. The MICs of INH and RF dropped. A detailed analysis of the results reveals that 17 MDR and 6 susceptible strains of *M. tuberculosis* showed additive effect with INH and ISO combination with FIC index 1. While two MDR and two susceptible strains showed synergistic effect with FIC index 0.5. While one MDR strain showed synergistic effect with FIC index 0.375 and two susceptible strains showed synergistic activity with FIC index 0.25 and 0.187.

While *M. scrofulaceum, M. flaevescens* and *M. kansasi* strains showed synergistic activity with FIC index of 0.5, 0.5 and 0.375 respectively. *M. fortuitum* strain showed additive effect. 18 MDR and 8 susceptible strains of *M. tuberculosis* showed additive effect with RF and ISO combination with FIC index 1. While two MDR strains and one susceptible strain showed synergistic effect with FIC index 0.5.While one susceptible strain showed synergistic activity with FIC index 0.25. While *M. scrofulaceum strain*showed synergistic activity with FIC index 0.5 and. *M. fortuitum, M. flaevescens* and *M. kansasi* strains showed additive effect.

The explanation for this apparent synergistic interaction between ISO & INH and ISO & RF in the face of resistance to all is unclear.

The mechanism of action of ISO against Mycobacteria is inhibition of mycolic acid synthesis. [13] As ISO affects cell wall in *M. tuberculosis*; this disruption allows INH & RF to gain access to the drug resistant and susceptible cells. This impermeability might be playing an important role in the resistance to drugs. Alternatively, enough minor cell wall disruption may be caused by INH, despite in vitro resistance to allow for the penetration of ISO into cell, when would normally be excluded. Indeed RF has been observed to enhance ISO activity. The hypothesis presupposes that impermeability is the mechanism of resistance to ISO in *M. tuberculosis*. This may be the case, since ISO apparently penetrates the envelope of other Mycobacteria to degree sufficient to its inhibition.

There is no literature available on combination study of ISO and RF in vitro and in vivo. While there is scarce literature available on combination study of ISO and other antitubercular drugs like INH, PAS and SM in vitro. Rosenweig D.Y [14] study compared efficacy of ISO in combination with INH in experimental TB in rabbits. ISO did not prevent death of some animals during first phase of treatment; on the other hand it was far more effective with INH alone under the same conditions. These results were confirmed by the clinical experiences of some authors**.** [15] Though some of the clinical trial studies with ISO and INH combination in past supports the synergistic activities of these two drugs. [16]It was reported that with combined INH and ISO treatment mean conversion rate of 91 % after 4 to 6 months, was found in more than 200 previously untreated cases. Rink et al (1967) [17] argued that there was only combination from INH and ISO left, which will be tolerated over long time by patients.

It was shown that combined chemotherapy with INH and ISO shows slightly better results than the monotherapy of INH. The long term results were expected to be better in this group because the combined therapy delays the development of Mycobacterial resistance and allows treatment to be continued until the primary lesions was under control. This is why a reactivation of latent primary foci was seldom to be seen, if it happens at all. There were no toxic side effects detected here. [15]

It appears that ISO had biological effect in the prevention of emergence of resistant strains, comparable to other standard drugs and as a corollary, must be efficacious as an antitubercular drug in combination therapy. [18]

CONCLUSION:

When INH & ISO and RF & ISO were used in combination against resistant and susceptible strains of *M. tuberculosis* and MOTT strains, enhancement of inhibitory activity of INH and RF was observed. The

VOLUME-6 | ISSUE-1 | JANUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

MICs of INH dropped more than RF along with ISO against resistant and susceptible strains of *M. tuberculosis* and MOTT strains. There was an in vitro synergistic interaction between ISO & INH and ISO & RF to *M.tuberculosis* and MOTT strains.

ISO and INH combination was more synergistic than ISO and RF combination.

It appears that ISO had biological effect in the prevention of emergence of resistant strains, to standard drugs and as a corollary, must be efficacious as an antituberculosis drug in combination therapy.

Acknowledgment:- Author acknowledges Mr. Jinesh Maniar & Ms. Dhanashree Deogharkar for providing inputs in preparation of manuscript.

REFERENCES:

1. Jawetz, E. (1968): The use of combinations of antimicrobial drugs. Ann. Rev. Pharmacol...8: 151.
2. Selkon, J.B., Devadatta, S., Kulkarni, K.G., Mitchison, D.A., Narayana, A.S.L., Nair, C.N. and Ramachandran, K. (1964): Bull Wrld. Health Organ. 31:273-294
3. Smith, C.B., Dans, P.E., Wilfert, J.N. and Finland, M.J. (1969): Infect Des. 119:370-377
4. Lehr, D. (1945): Proc. Soc. Exp. Biol. Med. 58:11-14.
5. Lau, W.K., Young, L.S., Black, R.E., Winston, D.J., Linne, S.R., Weinstein, R.J. and Hewitt, W.L. (1977): Am J Med. 62:959-966
6. Cohen, S.N. (1975): Combinations of Antibiotics – An introductory review. In Klastersky J (Ed): Clinical use of combinations of antibiotics. Wiley Biomedical Pub, New York: 1-10.
7. Simmons, N.A. (1980): Combinations of Antibacterial Drugs. In Gruneberg RN (ed): Current status of modern therapy, Vol.4 Antibiotics and Chemotherapy.:157-191
8. Young, L.S. (1984): Antimicrobial synergism and combination therapy. The Antimicrobic Newsletter. 1: 1.
9. Franzblau,S.G., Witzig, R.S., McLaughlin, J.C., Madico, T.P., Hernandez, G., Michelle, A., Degnan, T., Cook, M.B., Quenzer, V.K., Fergusan, R.M. and Gilman, R.H. (1998): Rapid low–technology MIC determination with clinical Mycobacterium tuberculosis isolates by using Microplate alamar blue assay. Jr. of Clin.Microbio.36: 362-366.
10. Krogstad, D. J. and Moellering, R.C. Jr. (1986). Combinations of antibiotics, mechanisms interaction against. In Lorraine V. 2nd Ed: Antibiotics in laboratory medicine. William and Wilkins, Baltimore M.D: 537-95.
11. Siddiqui, S.H., Libonati, J.P. and Middlebrook, G. (1981): Evaluation of a rapid radiometric method for drug susceptibility testing of Mycobacterium tuberculosis. Clin. Microbiol. 13:908-912.
12. Elion, G.B., Singer, S. and Hitchings, G.H. (1954): Antagonists of nucleic acid derivatives, VIII. Synergism in combinations of biochemically related antimetabolites. J. Biol. Chem. 208: 477
13. Winder, F. G., Collins, P.B. and Whelan, D. (1971): Effects of ethionamide and isoxyl on mycolic acid synthesis in Mycobacterium tuberculosis BCG. J. Gen. Microbiol. 66:379-380.
14. Rosenweig, D.Y. (1979): Pulmonary mycobacterial infections due to M.avium-intracellualarae-scrofulaceum complex .Chest.75:115.
15. Schutz, J. (1970): Discussion on isoxyl. Antibiotica ET Chemotherapia. 16:187-202.
16. Schmid, CH. (1970): Clinical experience in cases of primary tuberculosis with tuberculostaticum isixyl. Antibiot chemother. 16:108-116.
17. Rink, H. (1967): Uber den derzeitigen stand der Chemotherapie bei ambulanter Anwendung.Z.Tuberk. 126:1.
18. Gallen C.S.: (1970): Isoxyl: A review of the results of its use over a five year period in the Tuberculosis field service of large urben area. Antibiotica ET Chemotherapia. 16: 139-148.