Discussion

Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA) and Ethambutol (ETH) are first line drugs used for the treatment of tuberculosis. Streptomycin is also of the interest of physicians but not used now a days because of its injectable way of administration. The idea to study the effect of antitubercular drugs in terms of liver and kidney related changes perceived, as the tuberculosis in Indian population cover a wide range of population, particularly in the people with factors like malnutrition and poor hygienic conditions of living. Sometimes it has also been seen that disease has been found precipitated in people even in those who live in healthy conditions of life. The line of treatment is common to all and we have described above drugs are used in combination of either three or all four.

Rifampicin has bactericidal activity against mycobacterium tuberculosis by inhibiting bacterial DNA dependent RNA Polymerase while Isoniazid is a prodrug activated by bacterial Catalase-peroxidase (KATG) and kills actively growing tubercle bacilli by inhibiting the biosynthesis of mycolic acids which are major component of cell wall of Mycobacterium Tuberculosis.

The other drug Pyrazinamide is first activated by bacterial Pyrazinamidase which is only active in acidic conditions (PH 5.5). The active metabolite is pyrazinoic acid, which inhibits fatty acid synthesis in Mycobacterium Tuberculosis. Most of the time Pyrazinamide is used in first two months of the treatment and is not used alone.

On the other hand Ethambutol inhibits the synthesis of sub-
metabolite in actively growing Mycobacterium Tuberculosis causing impairment of cell metabolism, arrest of multiplication and cell death.

Drugs are not used solely in the treatment of tuberculosis instead the first line drugs are used in combination with other medicines. The single use of any drug may result in rapid development of resistance or failure of treatment. Several adverse reactions of antitubercular drugs are reported.

The best known toxic drug effect is hepatotoxicity. The frequency and severity of hepatotoxicity is found increased when these drugs are used in combination. These drugs are found as the promoters of CYT P450 enzymes for e.g Rifampicin is a good inducer of CYP 2D6 and CYP3A4 and Isoniazid induces CYP 2E1. It has been found in the earlier phase of our Hospital survey that patients also developed the repeated episodes of acute pain in joints and in such patients the level of serum uric acid was found elevated. The prescribing of Allopurinol to such patients by the physicians drew our attention to understand the beneficial as well as toxic effects of Allopurinol, also particularly in the patients those were already on the antitubercular drug treatment. Since all the drugs used in the treatment of tuberculosis are shown to have hepatotoxic effects other than a less percentage of renal toxicity and gouty effects. Our study was planned to understand the extent of toxicity created by these drugs individually and in combinations. The optimum dose of each drug was selected and the optimized drug alone and also in
combination was given to experimental animals.

Since Vitamin E and C are found good in liver fibrosis and other liver diseases, we understand the pathogenesis of liver damage usually depend on the underline disease. Oxidative damage of biologically relevant molecules making a common link between chronic liver injury and hepatic fibrosis. Hence use of Vitamin E supplementation is found beneficial.

We have controversial reports also, those show increased serum level of alpha-tocopherol following Vitamin E supplementation not always result in a protective effect. Therefore we also tried to set a firm role of Vitamin E and C supplementation particularly to control the extent of hepatotoxicity by antitubercular drugs and also control the negative effects of Allopurinol as we found on kidney.

Allopurinol is generally considered to be as a safe and well tolerated drug in treating hyperuricemia. 53% of patients receiving Allopurinol 300mg daily, achieve optimum plasma urate concentration, it is a thought of interest to see whether Allopurinol can control the increased levels of uric acid. The drug not only cause the positive response but can cause adverse reactions like itching, skin rashes, low grade fever, eosinophilia and life threatening adverse effects of acute renal damage. We also wish to see that the concentration of renal parameters is increased by the concurrent use of allopurinol and anti-tubercular drug treatment.

The development of adverse reactions of Allopurinol are due to serum levels of oxypurinol which is the therapeutic metabolite of
allopurinol. Oxypurinol inhibits Xanthine oxidase and preventing the conversion of Hypoxanthine and Xanthine to uric acid. Since the clearance is dependent on renal excretion the adverse reactions are included impaired renal functions.

We are hereby presenting our data for the individual drug in the first phase and in the combination in the second phase.

2.5mg/kg body wt. of the Isoniazid was found not to change any parameter up to a significant level. A little significance is reported in a level of serum Alkaline Phosphatase at 14th day of investigation which could not continue on 21st and 28th day respectively. Therefore this single significant increase cannot be taken into consideration with 2.5mg/kg body wt. of Isoniazid (Table I).

The results were overwhelming when Isoniazid was given in 5mg/kg body wt. dose. The levels of all the liver parameters were significantly increase when Isoniazid was given consequently for 30 days in a dose of 5mg/kg body wt. Serum Alanine Transaminase was found highly significant from the 2nd week of drug administration. Its level remained significantly increase up to the end of drug administration. Serum level of Alkaline Phosphatase showed significantly increase by the end of 1st week of drug administration. It also remain significantly high up to the end of drug feeding. Serum Bilirubin also showed a presentable increase from the 1st day only. Its level shows slightly significantly increased on the 1st day and become highly significant by the end of 1st week (Table II).

When a higher dose of 10mg/kg body wt. of Isoniazid was given
the serum levels of Alanine Transminase, Alkaline Phosphatase and Bilirubin showed a positive pattern but a less significant data was obtained for e.g Alanine Transminase is slightly significant in the last week of drug administration, Alkaline Phosphatase also found less significant on the 7th and 14th day and highly significant on the 21st and 28th day. The Bilirubin is showing a less degree of significance even after 3 weeks of drug administration and become highly significant only in the last week of drug administration (Table III).

The levels of Blood Urea, Serum Uric Acid and Serum Creatinine showed no significant change in their concentrations in any of the dose pattern (Table III), therefore we concluded that a dose of 5mg/kg body wt. of Isoniazid is a good dose for further experiments and we concluded it as an optimum dose of Isoniazid (Table II).

Another set of experimental animals was used to optimize the dose of Rifampicin. Rifampicin in dose of 5mg/kg body wt. could increase Serum Alanine Transminase only in the last week with a very less degree of significance. Serum Alkaline Phosphatase remain unaltered up to 21st day and increased significantly only in the last week of drug administration. Serum Bilirubin is increased after two weeks but the collective data is of moderate significance (Table IV).

The dose of 10mg/kg body wt. of Rifampicin also showed a similar pattern of data as it was with 5mg/kg body wt of Rifampicin, but an interesting result was that Blood Urea level is increased by the end of 2nd week and continued till the end of experiment with a lesser degree of significance. The results are having a new approach that so
far it is reported that Rifampicin is causing only hepatotoxicity but here an increased level of urea is found even when it is not highly significant, and is showing a scope of further investigation with a higher dose of Rifampicin (Table V).

When animals were given 20mg/kg body wt. of Rifampicin the Serum Alanine Transaminase level increased by the 3rd week significantly and highly significant by the end of 4th week. Serum Alkaline Phosphatase and Bilirubin started increasing and were found highly significant by the end of 2nd week of treatment. The level of Blood Urea and Serum Creatinine were also found significantly increased after 3 weeks of drug administration. So here we can conclude that Rifampicin is causing a good amount of hepatotoxicity and a less amount of renal toxicity when given in 20mg/kg body wt dose. This dose was selected as the optimum dose for the further set of experiments (Table VI).

12.5mg/kg body wt. of Pyrazinamide showed a significant data of Serum Creatinine after the three weeks of drug administration. The levels of Serum Alanine Transaminase, Serum Alkaline Phosphatase, Serum Bilirubin, Blood Urea and Serum Uric acid remain unaltered during the entire course of 12.5mg/kg body wt. of Pyrazinamide (Table VII).

25mg/kg body wt. of Pyrazinamide caused a good amount of Renal toxicity in the next set of experiments. Blood Urea started increasing after two weeks and became significant in the third week and highly significant in the fourth week. Serum Uric Acid also
increased significantly after one week and became highly significant by the end of 4th week. Serum Creatinine is also increased by the end of 1st week and became significant in the 3rd week and highly significant in the 4th week (Table VIII).

On the other hand the 50mg/kg body wt. of Pyrazinamide is not showing any significant increase therefore a thought is provoked that can we avoid all toxic effects of Pyrazinamide if it is used in a high dose (Table IX). However a dose of 25mg/kg body wt. was selected for the further set of experiments.

7.5mg, 15mg, 30mg doses of Ethambutol were used in different group of animals, but no significant change was found either in liver parameter or on kidney parameters therefore Ethambutol experiments were not performed further, neither exclusively nor in combination (Table X, XI, XII).

The liver is highly susceptible to predictable and unpredictable adverse effects. Unpredictable adverse reactions seem to occur in certain individuals leading to hepatic injury by either allergic or an abnormal drug metabolic mechanism.

Our experiments related to all primary drugs of tuberculosis showed a good involvement of hepatic toxicity. As we have discussed earlier that Pyrazinamide is causing a temporary rise in Serum Uric Acid content, and it is an obvious interest to see the effect of Allopurinol on liver and kidney in the fresh group of animals and in the animals those were given 25mg/kg body wt. of Pyrazinamide one month earlier.
Allopurinol when given in a dose of 0.75mg/kg body wt. only Blood Urea shows a significant increase on the 28th day of experiment, rest all biomarkers however shows a increase in level but that increase was not found significant (Table XIII). When Allopurinol was given in a dose of 1.5mg/kg body wt. Serum Alanine Transminase shows a non significant increase from the day 14th itself. Same results were obtained for the Serum Alkaline Phosphatase which shows a non significant increase from the day 21 only. Serum Bilirubin and Serum Uric Acid shows no significant change in their level when compared with control. However, Blood Urea and Serum Creatinine shows a significant increase on the 28th of the experiment (XIV). Allopurinol with a dose of 3mg/kg body wt. shows a non significant increase of the liver enzymes (Serum Alanine Transminase and serum Alkaline Phosphatase). Serum Bilirubin does not show any significant change throughout the experiment. Blood Urea and Serum Uric Acid shows a highly significant change on day 14th and very highly significant change was observed on day 21 and day 28th. Serum Creatinine increase was highly significant, after one week and in the 2nd, 3rd and 4th week it shows very high significance levels when compared with control (Table XV).

Since hepatic adverse effects of Allopurinol are rare, such reactions in susceptible host are probably immunologically mediated. 3mg/kg body wt. dose of Allopurinol showed a marginal increase in liver enzymes but not up to the level of it significant data. But Blood Urea, Serum Uric Acid and Serum Creatinine all indicators of renal
impairment showed a high significant increase in the 2nd week of Allopurinol treatment and data is very highly significant in the 4th and final week of the drug administration. We continued the experiments of Allopurinol upto 90 days with a dose of 3mg/kg body wt. and no change in the pattern was obtained. Renal biomarkers and Erythrocyte Sedimentation Rate are significantly increased after four weeks of treatment and data continued to be highly significant even after the completion of experiment up to eight weeks time (Table XIX).

Once the doses of Isoniazid, Rifampicin and Pyrazinamide were optimized for further set of experiments. The concurrent experiments with individual drugs were performed with 10 animals in each group with continuation of optimized doses for 12 weeks. It was surprising that the data showed in all the drugs is almost like the dose optimization pattern. Serum Alanine Transaminase, Serum Alkaline Phosphatase and Serum Bilirubin remain significantly increased up to the end of experiments with Isoniazid 5mg/kg body wt. dose, a highly significant increase was also observed in Erythrocyte Sedimentation Rate. A significant change has been observed in Blood Urea and Serum Creatinine in the last week of our treatment (Table XVI). Since we have noticed already the effect of Rifampicin on liver and kidney both. Therefore a significant effect on liver function on prolonged administration of Isoniazid is also not surprising.

20mg/kg body wt. dose of Rifampicin showed a very high significance in Serum Alanine Transaminase after 4 weeks of treatment while Serum Alkaline Phosphatase and Serum Bilirubin are
significantly increased after two weeks and became highly significant after 4 weeks like Serum Alanine Transminase. Blood Urea is also found elevated after two weeks and became very highly significant after two months. Serum Creatinine is also increased but not as high as Blood Urea and Serum Uric Acid showed a significant change in 90 days experiment. On the other hand Erythrocyte Sedimentation Rate is increased significantly after 45 days and become highly significant after two months and become very highly significant up to the end of experiment of 90 days (Table XVII). Therefore we can conclude that Isoniazid and Rifampicin are showing almost identical behavior that Isoniazid is hepatotoxic and renal toxic on prolonged administration. Rifampicin is also hepatotoxic and renal toxic up to a higher limit on prolonged treatment.

Pyrazinamide in 25mg/kg body wt. dose is not causing any toxicity in liver, though a significant increase in Serum Bilirubin has been reported after two months of treatment, but on the other hand Pyrazinamide show a good toxic effect on renal parameters. Blood Urea and Serum Creatinine are increased after one month and became very highly significant by the end of three months treatment. Serum Uric Acid also showed a significant data after 1 week and became very highly significant after 4 weeks in drug optimized experiment of Pyrazinamide but when the dose is continued for a longer time then Serum Uric Acid is very highly significant after 8 weeks of drug administration (Table XVIII).

We understand that the drugs are applied in combination of
two, three and four. Sometimes all four drugs are started in the beginning and one or two drugs withdrawn after two months of administration. Therefore we also planned the experiments of Isoniazid 5mg/kg body wt. dose and Rifampicin 20mg/kg body wt. dose in one group (**Table XX**) and Isoniazid 5mg/kg body wt. dose, Rifampicin 20mg/kg body wt. dose and Pyrazinamide 25mg/kg body wt. dose were taken in 2\textsuperscript{nd} set of experiments (**Table XXI**). We did not include Ethambutol as no significant effect was seen on liver and kidney with this drug, when used even in three different doses.

In the first set of experiments with Isoniazid and Rifampicin, the received data is of the nature of expectations that Serum Alanine Transminase, Serum Alkaline Phosphatase and Serum Bilirubin are increased and very highly increased up to the end of experiment with these two drugs, kidney toxicity was very high with Rifampicin alone in 20mg/kg body wt. dose and a slight significant change with Isoniazid alone by the end of experiment. But when these two drug are applied in combination only urea showed a significant increase after two months and could not reach up to the level of very highly significant even after the completion of experiment of 90 days. Erythrocyte Sedimentation Rate which was found increase by both the drugs individually, showed an increase after two months and became high significant after 10 weeks and highly significant at the end of experiment (**Table XX**). Therefore it has been noticed that when these two drugs are used in combination then extent of renal toxicity is limited.
Now it has again become very important to include Pyrazinamide in the combination. In this set of experiment Serum Alanine Transaminase, Serum Alkaline Phosphatase, Serum Bilirubin, Blood Urea and Serum Uric Acid along with Erythrocyte Sedimentation Rate all increased very highly significant by the end of 90 days. Erythrocyte Sedimentation Rate also increase but only after two months and reached very highly significant increase like the other parameters in the end of experiment (Table XXI). The toxic effects of Pyrazinamide are to penetrating that the withdrawal of Pyrazinamide after two weeks is seems to be logical.

We have noticed that now a days a good stressed is given by the biochemists and biotechnologist all over the world on the role of antioxidants. The generation of free radicals is considered to be an important cause of many age related and enviromental clinical disorders and overcoming of these disorders by antioxidants is reported almost in every research journal from last five years. Therefore we also thought the weather any good antioxidant can control the toxic effects created by our experimental drugs.

The selection of Vitamin C and E was dependent on their qualities.

**VITAMIN C**

- Vitamin C has a biological role as a reducing agent in hydroxylation reactions in the body.
- Vitamin C is structurally similar to glucose.
- Vitamin C has antioxidant activity.
Vitamin C helps in absorption of iron by reducing it to ferrous state in the stomach.

- 80-90% of Vitamin C is absorbed.
- Vitamin C is easily oxidized.
- Vitamin C works as a cofactor for many enzymes.
- Being a water soluble antioxidant can curtail both reactive oxygen and reactive nitrogen, reduces lipid peroxidation, oxidative DNA damage and oxidative protein damage, reduce oxidation of LDL, increase HDL.

- Vitamin C can prevent the formation of carcinogen.

**VITAMIN E**

- Vitamin E is a fat soluble antioxidant.
- Vitamin E protects polyunsaturated fatty acids from oxidation by destroying peroxyl radicals.
- Vitamin E inhibits the activity of protein kinase C.
- Vitamin E inhibit platelet aggregation.
- Vitamin E may be involved in signal transduction in neuronal cells. Since it is associated with neuronal cell membranes and other lipids in the nervous system.

Therefore it seems to be logical to see the effects of these two vitamins to overcome the toxicity by antitubercular drugs and allopurinol.

Vitamin C was given in 120mg/kg body wt. dose along with
Isoniazid, Rifampicin and Pyrazinamide to experimental animals consecutively for three months daily and blood samples were collected on 1, 15, 30, 45, 60, 75 and 90 days respectively. We are surprised that the pattern of table is almost same as it was earlier along with Isoniazid, Rifampicin and Pyrazinamide without the involvement of Vitamin C. A good amount of toxicity is reported in terms of Serum Alanine Transaminase, Serum Alkaline Phosphatase, Serum Bilirubin, Blood Urea, Serum Uric Acid and Serum Creatinine all are very significantly increased in the end of experiment. Probably the role of Vitamin C is non-effective in the presence of these three primary drugs (Table XXII). The adverse effects of Allopurinol were also remain uncontrolled when Vitamin C was given along with 3mg/kg body wt. of Allopurinol for 90 days (Table XXIV).

The effect of Vitamin E in 200mg/kg body wt. dose is very much appreciable as it totally behaved oppositely to the effects of Vitamin C. When Vitamin E is given along with Isoniazid, Rifampicin and Pyrazinamide the concentration of Serum Alanine Transaminase decreased significantly after 10 weeks and very highly significant after 90 days. Serum Alkaline Phosphatase also decreased significantly by the end of experiment the concentration of Serum Bilirubin, Blood Urea and Serum Uric Acid also decreased in the end of experiment. The concentration of Serum Creatinine was also controlled. We did not notice any effect of Vitamin E supplementation on Erythrocyte Sedimentation Rate (Table XXIII).

When Allopurinol with a dose of 3mg/kg body wt. is treated with Vitamin E 200mg/kg body wt. it shows a significant decrease in the
Serum Alanine Transminase from the day 60 up to the end of experiment, when compared with control. Similarly Serum Alkaline Phosphatase shows a significant decrease on day 90 when compared with control. However Serum Bilirubin shows a highly significant decrease from day 75 when compared with control. Blood Urea was found to be highly significant decrease on the 90th day of the experiment. Serum Uric Acid also shows a decrease in the level but that decrease was not significant. Serum Creatinine on day 60 shows a significant decrease and very highly significant decrease was found on day 75 on day 90. There was a decrease in Serum Creatinine level but that decrease was not significant. Erythrocyte Sedimentation Rate shows a significant increase from the day 45 only, when compared with control (Table XXV).

Since ASO Titre and RA Factor is found to be negative in all doses of different drugs either alone or in combination, despite of having significantly increased Erythrocyte Sedimentation Rate and Serum Uric Acid level. This is suggesting that there is some infection but not the chronic one.

The increased level of Erythrocyte Sedimentation Rate is suggestive that experimental animals were having any infection earlier or developed an infection because of the toxic effects of the drugs. So it is concluded that-

1. If possible the drug Pyrazinamide should be used for a limited time.
2. A vitamin E supplementation should be given along with antitubercular treatment.