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Research Article

Effect of α-tocopherol on antitubercular drugs induced hepatotoxicity

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ABSTRACT

Background: *Mycobacterium*, the causative organism of tuberculosis, is notorious for its ability to develop resistance with monotherapy. To prevent emergence of resistance, combination of antitubercular drugs is given for months to years that can lead to side effects. Hepatotoxicity is one of the commonest side-effect with antitubercular drugs. This study was aimed to explore the hepatoprotective potential of α-tocopherol against experimentally induced hepatotoxicity in albino rabbits.
Methods: This experimental study was carried out on 30 rabbits of either sex. They were divided into three groups comprising 10 animals each. Hepatotoxicity is induced experimentally in rabbits following a standard protocol. Group I received normal saline (10 ml/kg bw). Rabbits in group II were treated with first line antitubercular drugs isoniazid (5 mg/kg bw), rifampicin (20 mg/kg bw) and pyrazinamide (25 mg/kg bw) concurrently. Group III received α-tocopherol 200 mg/kg bw along with group II drugs. All drugs were administered by oral route for 90 days. On last day of experiment blood samples were taken to investigate the plasma levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum total bilirubin.
Results: Serum levels of ALT were found to be markedly elevated upon oral administration of antitubercular drugs for 90 days. A statistically significant reduction in ALT levels was noticed when α-Tocopherol was given in doses of 200mg/kg bw along with antitubercular drugs for same duration. Similar results were obtained with serum ALP & serum total bilirubin.
Conclusions: α-tocopherol (200 mg/kg bw, oral) was found to have hepatoprotective effect against antitubercular drugs induced hepatotoxicity in albino rabbits.

Keywords: Antitubercular drugs, Hepatotoxicity, α-tocopherol, Antioxidents

INTRODUCTION

India has the highest burden of tubercular patients worldwide. As per WHO statistics nearly two million people develop active tuberculosis per year and about 1000 people die from tuberculosis everyday.[1]

Isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (ETH) are first line oral antitubercular drugs. The single use of any first line drug can lead to the rapid development of drug resistant tuberculosis that is very difficult to treat. Therefore first line antitubercular drugs are used in combination as per WHO recommendation.[2]

Hepatotoxicity is the most well known toxic effect with antitubercular drugs. Any one or more of INH, RIF or PZN could be causative especially when these drugs are used as combination therapy as per standard protocol.[3] It has been reported that the generation of free radicals is associated with toxic effect of antitubercular drugs.[4] This study was aimed to investigate the possible effect of α-

tocopherol (antioxidant) in protecting hepatotoxicity of first line oral antitubercular drugs in rabbits.

METHODS

This study was conducted in Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar as well as in Department of Pharmacology, LLRM Medical college, Meerut, Uttar Pradesh, India from March 2008 to September 2009.

Animals

Albino rabbits of either sex weighing 1-3 Kg were procured from central animal house of the institute and were housed individually in cages, in air conditioned environment. They were provided with food and water *ad libitum*. Study was begun following an acclimatization period of 7 to 10 days.

Drugs

Isoniazid and rifampicin (powder dosage form) gifted by Lupin Research lab Ltd. α-tocopherol (Vit E) was gifted by Franco India Pharmaceuticals Ltd. Pyrazinamide and other used chemicals were provided by Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar, India.

Doses and route of administration

Each drug was administrated in three different doses in the early stage of experiment, to select the optimum dose that was found as INH 5mg/Kg bw, RIF 20mg/Kg bw, PZN 25mg/Kg bw.

Selected optimum doses of first line oral antitubercular drugs were administrated concurrently to match the pattern of administrated drugs in tubercular patients. Ethambutol was excluded from the study because it is not hepatotoxic.[5] Route of administration was oral.

Study design

Following approval from Institutional animal ethics committee the study was carried out from March 2008 to September 2009. Thirty albino rabbits of either sex were used and were divided into three groups with 10 animals in each group. They were treated with drug for three months. Doses were administrated per Kg body weight, by oral route, as following.

- Group I (control group)- Normal saline (10ml/Kg bw)[6]
- Group II- INH (5mg/Kg bw) + RIF (20mg/Kg bw) + PYZ (25mg/kg bw)
- Group III-INH (5mg/Kg bw) + RIF (20mg/Kg bw) + PYZ (25mg/kg bw) + Vit. E (200mg /Kg bw)

Blood sample were taken before drug administration at day 1 and then at weekly interval till the end of study.

Serum was separated from blood sample by centrifugation at 2500 rpm and stored at refrigerated temperature till analysis (2 to 4 weeks) was done. Serum was assayed for the levels of all biochemical parameters.

Serum levels of ALT, ALP and total bilirubin were measured by using commercially available kits.

Statistical analysis

Results were presented as mean and standard deviation of mean. Statistical significance was determined at the $P<0.05$, 0.01, 0.001. Intra group comparisons were made for the levels of biomarkers with control and statistically evaluated by student "t" test.

RESULTS

The mean serum AST level of the control group was 29.10±3.30 IU/L before initiating administration of normal saline in doses of 10mg/Kg bw. No significant changes in this value were observed till the end of the experiment. Similarly the serum level of ALP and Total bilirubin remained constant as 120.10±7.21 IU/L and 0.15±0.02 mg/dl respectively from the day 0 to day 90. Thus administration of normal saline by oral route did not show any change during the experiment.

Oral administration of a combination of INH 5mg/Kg bw, RIF 20 mg/Kg bw and PYZ 25mg/kg bw resulted in a significant rise in serum AST level from 28.60±3.38 IU/L at day one to 56.80±7.00 IU/L at day 90 in comparison to control group ($P<0.001$) (Table 1, Figure 1A).

Treatment with α-tocopherol along with INH+RIF+PYZ administration significantly reduced Serum AST level from 56.80±7.00 IU/L to 20.00±3.89 IU/L at day 90($P<0.001$) (Table 2, Figure 2A).

There was an increase in serum ALP in the INH+RIF+PYZ treated rabbits. The respective values in group I and group II were 120.10±7.21 IU/L and 183.00±7.79 IU/L (Table 1, Figure 1A). Administration of α-tocopherol along with INH+RIF+PYZ significantly reduced serum ALP level from183.00±7.79 IU/L to 112.00±6.60 IU/L. The difference was found statistically significant ($p<0.05$) (Table 2, Figure 2A).

The serum total bilirubin was 0.15±0.02mg/dl in group I. It was found significantly increased to 1.70±0.61 mg/dl at day 90 ($p<0.001$) in INH+RIF+PYZ treated group (Table 1, Figure 1B). Administration of α-tocopherol along with INH+RIF+PYZ significantly reduced total bilirubin from 1.70±0.61mg/dl to 0.10±0.03mg /dl at day 90. (Table 2, Figure 2B).

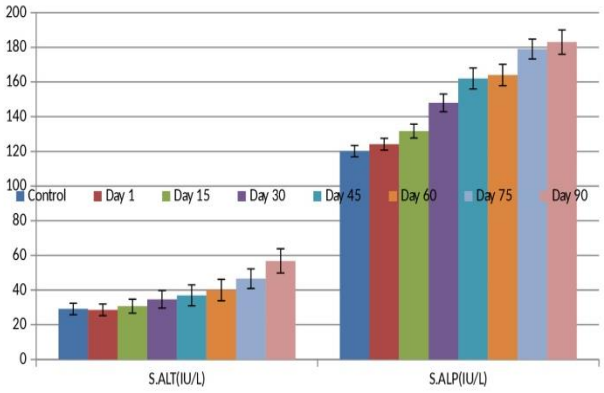

Figure 1A: Levels of ALT and ALP following administration of a combination of INH 5+RIF 20+ PYZ 25 mg/kg bw.

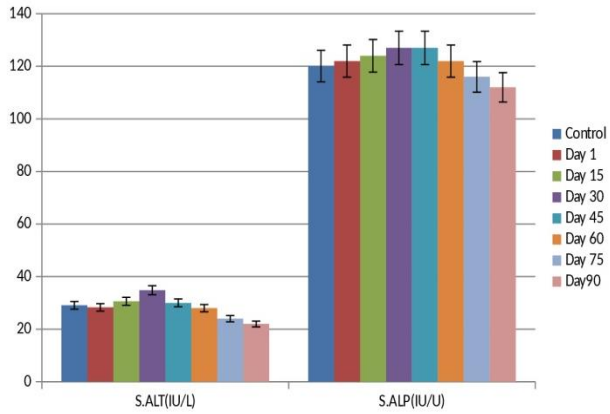

Figure 2A: Levels of ALT and ALP following administration of α tocopherol (200mg/kg bw) and combin ation of INH 5+RIF 20 + PYZ 25 mg/kg bw.

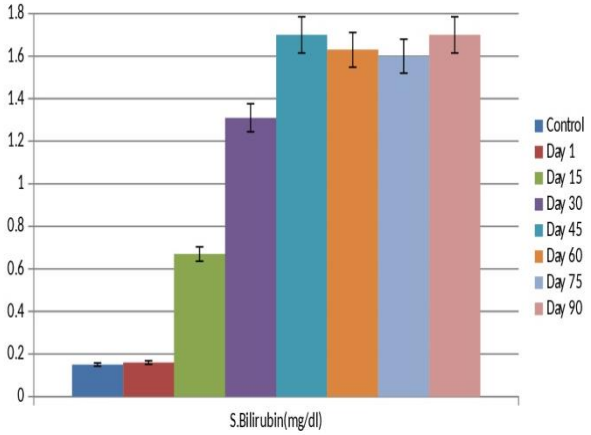

Figure 1B: Serum bilirubin levels following administration of a combination of INH 5+RIF 20 + PYZ 25 mg/kg bw.

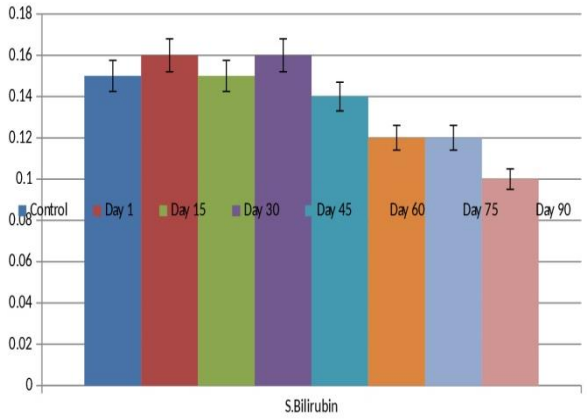

Figure 2B: Serum bilirubin level following administration of α tocopherol (200mg/kg bw) and combination of INH 5+RIF 20+PYZ 25 mg/kg bw.

Table 1: Levels of ALT, ALP and serum bilirubin following administration of a combination of INH 5+RIF 20 + PYZ 25 mg/kg bw.

Biochemical parameter	INH 5+RIF 20 + PYZ 25 mg/Kg Body Wt.							
	Control	Day 1	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
S. ALT (IU/lt)	29.10 ± 3.30	28.60 ± 3.38	30.70 ± 3.97	34.60* ± 5.10	36.90** ± 6.01	40.00*** ± 6.17	46.60*** ± 5.70	56.80*** ± 7.00
S. ALP (IU/lt)	120.1 ± 7.21	124.10 ± 7.24	131.7** ± 7.00	148.0*** ± 6.83	162.0*** ± 7.70	164.0*** ± 8.11	179.0*** ± 7.60	183.0*** ± 7.79
S. bilirubin (mg/dl)	0.15 ± 0.02	0.16*** ± 0.02	0.67*** ± 0.04	1.31*** ± 0.10	1.70*** ± 0.09	1.63*** ± 0.07	1.60*** ± 0.05	1.70*** ± 0.61

Values are expressed as their mean ± SD; * P<0.05; ** P<0.01; *** P<0.001.

Table 2: Levels of ALT, ALP and serum bilirubin following administration of α-tocopherol (200mg/kg bw) and combination of INH 5+RIF 20 + PYZ 25 mg/Kg bw.

Biochemical parameter	INH 5+RIF 20 + PYZ 25 + α Tocopherol 200mg/Kg Body Wt.							
	Control	Day 1	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
S. ALT (IU/lt)	29.10 ± 3.30	28.30 ± 3.23	30.60 ± 3.10	34.80* ± 3.71	30.00 ± 3.51	28.00 ± 4.00	24.00** ± 3.91	22.00*** ± 3.89
S. ALP (IU/lt)	120.1 ± 7.21	122.0 ± 8.30	124.0 ± 8.10	127.0 ± 7.80	127.0 ± 8.10	122.0 ± 7.86	116.0 ± 6.91	112.0* ± 6.60
S. Bilirubin (mg/dl)	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.14 ± 0.03	0.12** ± 0.02	0.12** ± 0.02	0.10*** ± 0.03

Values are expressed as their mean ± SD; * P<0.05; ** P<0.01; *** P<0.001.

DISCUSSION

Rabbit is a reliable, reproducible clinically relevant animal model of antitubercular drug induced toxicity.[7] Sarich TC et al reported rabbit a good and sensitive model of hepatotoxicity. The pattern of hepatotoxicity produced in rabbits resembles that of human.[8] In our study treatment was given orally, as oral route is the preferred route of administration of anti tubercular drugs. Monotherapy with antitubercular drugs can lead to rapid development of resistance resulting in multi drug resistance tuberculosis that is very difficult to treat. Therefore combination of anti-tubercular drugs was used in our study.[2] The best known toxic effect of first line oral anti tubercular drugs is hepatotoxicity.[9] Incidence is increased when these drugs are used in combination. INH, RIF, PYZ are the first line anti tubercular drugs having hepatotoxic potential.[10] Therefore this combination was used in our study to induced hepatotoxicity in rabbits. This fact is also reported by an earlier study by Durand F *et al*.[11] This study demonstrated that INH and PYZ are major hepatotoxic antitubercular drugs. The remaining two drugs (RIF and ETM) are rarely or not hepatotoxic. However, RIF, is a powerful enzyme inducer may enhance the hepatotoxicity of INH.[12]

All anti tubercular drugs treated rabbit showed elevated levels of serum hepatic biomarkers of hepatotoxicity ALT, ALP & total bilirubin. These findings support previous studies reported by Singhal KC *et al*.[13]

There are inadequate data regarding hepatoprotective effect of α-tocophherol in hepatotoxicity induced by anti tubercular drugs. So far, there are only few studies which investigated the effectiveness of α-tocopherol in INH, RIF and PYZ induced hepatotoxicity in experimental animals. A study by Skakun et al showed hepatoprotective potential of α- tocopherol on INH, RIF and PYZ induced hepatotoxicity in rats. The results of our study also support the finding of study by Skakun *et al*.[14]

Although the mechanism behind the hepatoprotective effect of α- tocopherol is not known. Electrophilic intermediates produced during metabolism of anti tubercular drugs are highly reactive free radicals. These free radicals may lead to oxidative stress resulting in cell injury or cell death by various mechanisms as lipid per oxidation.[16]

One possible mechanism for hepatoprotective effect of α-tocopherol may be its antioxidant property. α-topopherol was found to inhibit lipid peroxidation in hepatocytes caused by agents like carbon tetrachloride and halothane.[16,17] Thus α-tocopherol may act as antioxidant as reported by Martine ZC *et al* in their study that stated protective effect of α-tocopherol in halothane induced hepatotoxicity in rats.[17] Another study by Saraswathy SD *et al* also reported that treatment with antioxidant preparation such as Liv.100 offers protection against INH induced hepatotoxicity in rats by reducing lipid per oxidation and restoring the anti oxidant defense system .[18]

CONCLUSION

Thus it may be concluded that α-tocopherol has hepatoprotective potential against INH, RIF and PYZ induced hepatotoxicity in rabbits. Further studies on large sample size for longer duration are needed to explore hepatoprotective effect of α-tocopherol.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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