

## Prevention of hepatotoxicity due to anti tuberculosis treatment: A novel integrative approach

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### Abstract

**AIM:** To evaluate the ability of *Curcuma longa* (CL) and *Tinospora cordifolia* (TC) formulation to prevent anti-tuberculosis (TB) treatment (ATT) induced hepatotoxicity.

**METHODS:** Patients with active TB diagnosis were randomized to a drug control group and a trial group on drugs plus an herbal formulation. Isoniazid, rifampicin, pyrazinamide and ethambutol for first 2 mo followed by continuation phase therapy excluding Pyrazinamide for 4 mo comprised the anti-tuberculous treatment. Curcumin enriched (25%) CL and a hydro-ethanolic extract enriched (50%) TC 1 g each divided in two doses comprised the herbal adjuvant. Hemogram, bilirubin and liver enzymes were tested initially and monthly till the end of study to evaluate the result.

**RESULTS:** Incidence and severity of hepatotoxicity was significantly lower in trial group (incidence: 27/192 vs 2/316,  $P < 0.0001$ ). Mean aspartate transaminase (AST) ( $195.93 \pm 108.74$  vs  $85 \pm 4.24$ ,  $P < 0.0001$ ), alanine transaminase (ALT) ( $75.74 \pm 26.54$  vs  $41 \pm 1.41$ ,  $P < 0.0001$ ) and serum bilirubin ( $5.4 \pm 3.38$  vs  $1.5 \pm 0.42$ ,  $P < 0.0001$ ). A lesser sputum positivity ratio at the end of 4 wk (10/67 vs 4/137,  $P = 0.0068$ ) and decreased incidence of poorly resolved parenchymal lesion at the end of the treatment (9/152 vs 2/278,  $P$

= 0.0037) was observed. Improved patient compliance was indicated by nil drop-out in trial vs 10/192 in control group ( $P < 0.0001$ ).

**CONCLUSION:** The herbal formulation prevented hepatotoxicity significantly and improved the disease outcome as well as patient compliance without any toxicity or side effects.

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**Key words:** Hepatoprotection; Anti-tuberculous treatment; Curcumin longa; *Tinospora cordifolia*

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### INTRODUCTION

During the first few years of introduction of Isoniazid (INH), it was considered so safe that in 1963, The American Thoracic Society recommended for all tuberculin-positive persons to receive a year of INH chemoprophylaxis regardless of age or duration of the tuberculin positivity<sup>[1]</sup>. A retrospective study reported a 1% incidence of clinical hepatitis and a 0.1 % death in patients taking INH chemoprophylaxis<sup>[2]</sup>. A large multicenter prospective surveillance study by United States Public Health Service revealed a 1% incidence of hepatitis and 0.06% deaths from hepatitis due to INH<sup>[3]</sup>. After introduction of Rifampicin (RMP), several reports suggested that hepatitis was more frequent and severe in patients receiving both INH and RMP than in those receiving INH alone<sup>[4]</sup>. For preventing acquired

resistance and a successful treatment; it is recommended to start with a combination chemotherapy containing INH, RMP, and Pyrazinamide (PZA)-one more hepatotoxic agent- with or without ethambutol for the initial 2 mo followed by a continuation phase of 4-6 mo of INH + RMP<sup>[5]</sup>. It is well known that drug induced hepatotoxicity is a potentially serious adverse effect of anti-tuberculosis treatment (ATT) containing INH, RMP and PZA<sup>[6]</sup>. Preventive therapy of latent TB with 2-mo course of RMP and PZA has been associated with fatal and severe hepatotoxicity more often than does 6 mo of INH therapy or curative treatment of clinical TB<sup>[7-9]</sup>. Similar high risk was observed with PZA and ethambutol or a fluoroquinolone when given to contacts of multi-drug resistant TB patients<sup>[10]</sup>. Now integrating these observations with the fact that about one third of the world's population has latent TB and roughly 9 million cases of active TB emerge annually resulting in 2-3 million deaths<sup>[11]</sup>, exemplifies the magnitude and importance of the problem, especially when most new cases occur in the most populated nations like India and China<sup>[12]</sup>. Also, a higher risk of hepatotoxicity has been reported in Indian patients (up to 11.5%) than in their western counterpart (up to 4.3%)<sup>[4]</sup>. In a study of European patients, the incidence of ATT induced hepatotoxicity was found to be 18.2% in group having risk factors like, old age, extensive TB, malnutrition, alcoholism, HIV and chronic viral hepatitis B and C infections, as against 5.8% in group without risk factors indicating the significance of risk factors<sup>[13]</sup>.

The only measure available for managing ATT induced hepatotoxicity in clinical cases is stopping the offending agents, once there is an evidence of liver damage and reintroducing the same after normalization of liver enzymes<sup>[14,15]</sup>. To reduce the incidence of hepatotoxicity in latent TB patients, recommendations for drugs and patient selection criteria have been revised several times by organizations like Center for Disease Control, American Thoracic Society, Joint Tuberculosis Committee of British Thoracic Society, and Hong Kong Tuberculosis Service *etc.*, but until today no drug has been developed for prevention of hepatotoxicity.

The pathogenesis of hepatotoxicity is not entirely clear, but INH and RMP induced damage may involve oxidative stress<sup>[16]</sup>, lipid peroxidation<sup>[17]</sup>, choline deficiency leading to lowering of phospholipids protein synthesis with alteration in cell wall configuration<sup>[18]</sup>, reduced glutathione level<sup>[19]</sup> and activation of CYP2E1<sup>[20]</sup>. It is well known that some non-toxic herbs are having opposite activities in the form of membrane stabilizing, anti-oxidative and CYP2E1 inhibitory effects<sup>[21]</sup>. A review of available literature suggests that reduction in lipid peroxide content in tissue and increase in superoxide dismutase, catalase, glutathione, glutathione-S-transferase and glutathione peroxidase activities should help to maintain liver cell integrity and control the increase in level of liver enzymes.

In a previous preclinical study we found *Curcuma longa* (CL) and *Tinospora cordifolia* (TC) to offer protection in guinea pig model of ATT induced

hepatotoxicity<sup>[22]</sup>. Both these herbs have an excellent safe toxicological profile<sup>[23,24]</sup>. Phase-I clinical trials on curcumin showed that it is safe to humans up to 12000 mg/d when taken orally<sup>[25,26]</sup>. Several animal experiments for various activities of TC did not reveal any toxicity at dosage as high as 400 mg/kg while no adverse reactions have been noted in international adverse reaction database in spite of several clinical trials and wide spread usage in Ayurvedic system of medicine<sup>[27]</sup>. So it was logical and ethical to conduct a randomized controlled clinical trial to evaluate the efficacy of CL and TC to control the hepatotoxic episodes in patients diagnosed to have TB and undergoing ATT. The primary aim of the present study was to estimate to what extent CL and TC addition in the standard regime affects hepatotoxicity profile and the secondary being whether the well known immunomodulatory and tonic activity of these herbs have any impact on the outcome of TB itself. To the best of our knowledge, such a clinical trial using hepatoprotective herbs as an adjuvant medicine to prevent ATT induced hepatotoxicity has not been performed anywhere.

## MATERIALS AND METHODS

### Study population

All the cases between the ages of 15 to 85 years having evidence of pulmonary or extra-pulmonary TB requiring a full curative course of ATT and coming to the clinic run by Shree Gurudev Sarvajanic Charitable Trust (SGSCT) from April 2005 to March 2007 were screened for enrollment in the trial with reservations as shown in Table 1. As the six centers of the mobile clinic were attending patients on a weekly basis for any illness, catering more than 200 villages of the District Surat and adjoining state Maharashtra, the local primary care agencies were advised and allowed to give routine treatment for inter-current illnesses and patients were advised to inform about the episodes and treatment given.

As shown in Figure 1, out of 578 patients screened 528 were found eligible. Periodic review was done by an independent review committee at 48, 100, 148 and 200 patients' recruitment in each group. The interim analysis of first 400 patients performed in October, 2006 revealed zero incidence of hepatotoxicity in the trial as against 22 patients with any grade of hepatotoxicity and 16 patients with no improvement in functional status in control group. The trial was granted with an intention to treat and as the criteria for outcome assessment were objective and as the sample size exceeded the WHO criteria for adequacy of sample size with power > 80%, the control arm was truncated on ethical ground and later all the patients were recruited in trial arm till March, 2007; only to find out whether any case of hepatotoxicity was encountered.

During their baseline visit, patients underwent a review of symptoms that included history of nausea, vomiting, jaundice, abdominal pain, weight loss, arthralgia, headache and neuropathy.

Table 1 Protocol for selection of patients for recruitment

Protocol for selection of patients for recruitment
Criteria for selection
Evidence of active pulmonary or extra-pulmonary tuberculosis
Age between 15 and 85 years
Readiness to comply with randomization, treatment and follow-up protocols
Patients giving written informed consent
Absence of diseases warranting treatment with systemic steroids, antimetabolites or warfarin
Concomitant conditions allowed
Patients with relapse, multiple relapse, treatment failure of DOTS regimen
Patients with hypertension, diabetes mellitus, COPD (chronic obstructive pulmonary disease) with continuation of required medications
Patients with asthma on inhalational steroid
Skin conditions requiring topical small area steroid application
Patients with HIV positivity but without symptoms suggestive of AIDS
Hepatitis B/C virus carrier
Criteria for rejection
Presumptive diagnosis or lack of evidence of active tuberculosis
Age < 15 or > 85
Patients taking other alternative therapies for tuberculosis
Patients declining to give consent or comply with protocol
Pregnant females
Heavy alcoholism history, > 80 g of alcohol/d for males and > 20 g of alcohol/d for females <sup>[50]</sup>
Concomitant conditions not allowed
Preexisting liver disease with AST, ALT raised > twice upper normal, evidence of portal hypertension.
Preexisting renal disease with S. Creatinine raised > twice upper normal
Sickle cell disease with history of crisis, anemia and jaundice
History of gout
Recent drop-outs from other TB centers due to complications and side effects
Patients on steroid and/or antimetabolite for other collagen, autoimmune or neoplastic diseases

Table 2 Chemical constituents of formulation

Chemical constituents of formulation with active principles	
Chemical constituents of <i>curcuma longa</i> with active principles	
Curcuminoids	Desmethoxycurcumin Bisdemethoxycurcumin Curcumin
Turmerones	Ar-turmerone $\alpha$ -turmerone $\beta$ -turmerone
Curcumenes	$\gamma$ -Curcumene, ar-Curcumene, Dehydrocurcumene zingiberene $\beta$ -bisabolene $\beta$ -sesquiphellandrene
Miscellaneous	Terpinolene, <i>P</i> -Cymene, 1-8-Cineole, Curlone
Chemical constituents of <i>Tinospora cordifolia</i> with active principles	
Alkaloids	Berberine, Palmatine, Tembetarine, Magnoflorine, Choline, Tinosporin, Isocolumbin, Tetrahydropalmatine
Glycosides	18-norclerodane glycoside, Furanoid diterpene glucoside, Tinocordiside, Tinocordifolioside, Syringin, Syringin- <i>apiosylglycoside</i> , Palmatosides C, Palmatosides P Cordifolioside A, Cordifolioside B Cordifolioside- A, -B, -C, -D and -E
Diterpenoid Lactones	Furanolactone, Clerodane derivatives,
Steroids	Tinosporon, Tinosporides, Jateorine, Columbin $\beta$ -sitosterol, $\delta$ -sitosterol, 20 $\beta$ -hydroxycedysone Ecdysterone, Makisterone A, Giloinsterol.
Sesquiterpenoid Aliphatic compounds	Tinocordifolin Octacosanol, Heptacosanol, Nonacosan-15-one
Miscellaneous compounds	3, ( $\alpha$ , 4-dihydroxy-3-methoxy-benzyl)-4-(4-hydroxy-3-methoxy-benzyl)- tetrahydrofuran, Jatrorrhizine, Tinosporidine, cordifol, cordifellone N-trans-feruloyl tyramine as diacetate, Giloin, Giloinin

### Herbal formulation

By high performance thin layer chromatography (HPTLC) analysis of different samples, we found that Salem types of turmeric powder contained highest 4% of curcuminoids that are the main active molecules. In Ayurvedic literature, the dose for turmeric has been described to be 6-12 g/d<sup>[28]</sup>. Our patient population being malnourished with an average wt of 38 kg, we preferred equivalent of 6 g/d by enriching the turmeric powder curcumin content to 25% so that 1 g dose would suffice. The dose of *Tinospora cordifolia* is also described to be 6-12 g/d<sup>[29]</sup> and again we preferred to make 1 g/d equivalent of 6 g/d by enriching the crude *Tinospora cordifolia* with 10:1 strength hydro-ethanolic extract to 50%. The phytochemical groups with active principles of both the herbs are shown in Table 2<sup>[30-32]</sup> and HPTLC fingerprinting of the formulations used were done for standardization purpose and have been shown in Figure 2.

### Study design

Ninety percent of all the patients were tribal, either working as laborers or owner of small piece of

agricultural land, while 10% were belonging to suburban area and belonging to other casts and occupations. Hence patients were explained in their local language about the protocol and those agreeing to comply were assigned the group by permuted block randomization for a balanced distribution. Because the evaluation criteria were fully objective and as the biochemical assessments were done in laboratories at a distance by unknown technicians, patients were not blinded conventionally about the therapy. Being a joint project it was approved by both the institutional ethical committee and informed consents were obtained from all the patients.

All of the eligible patients in the control group were started with an intensive phase ATT comprising of INH 300 mg/d, RMP 450 mg/d and PZA 20 mg/kg body wt to the closest 250 mg in two divided doses and Ethambutol 800 mg HS for initial 2 mo and than three drugs excluding PZA for 4 mo. Similar ATT medications supplemented with curcumin enriched (25%) CL and a hydro-ethanolic extract enriched (50%) TC 1 g/d each in two divided doses started from day one in trial group. Other supplemental medications like vitamins, iron, H2 blockers, proton pump inhibitors, anti-emetics,

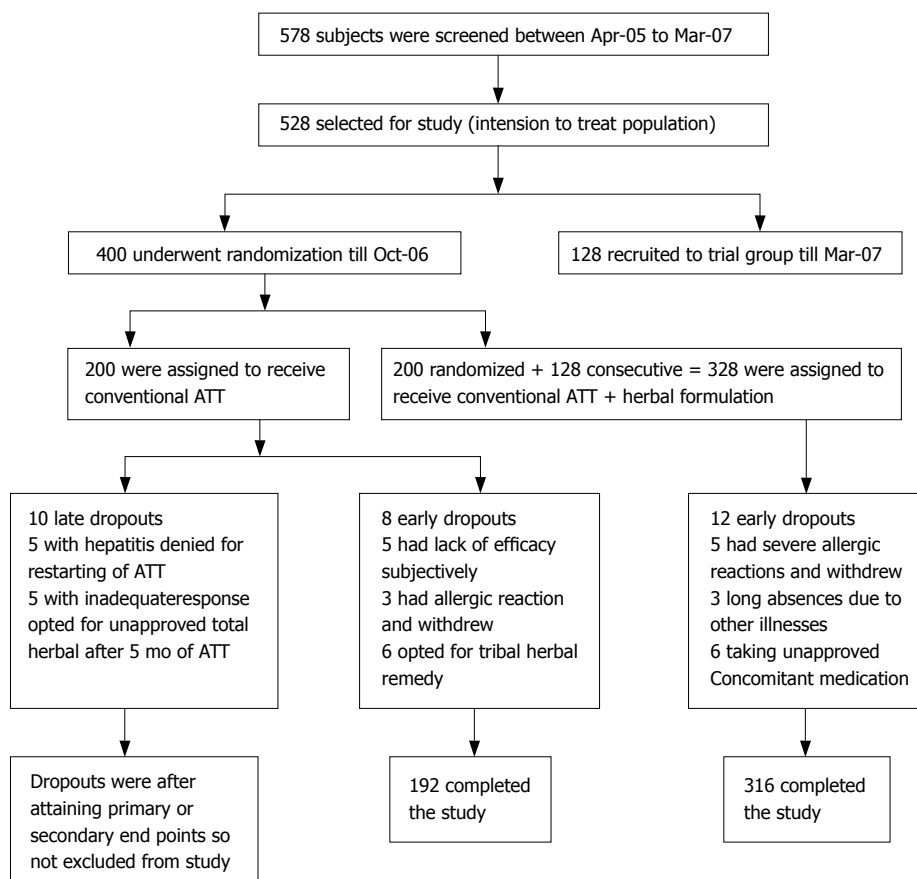


Figure 1 Study design and enrollment and follow-up of patients.

gut motility enhancers and calcium were also being given on an individual basis to patients in any group as deemed necessary by the physician. Two weeks quota of medications was given for self administration and patients were advised to return to the clinic every two weeks or earlier if any troublesome symptoms mentioned earlier were experienced. Blood sample for liver enzymes and bilirubin were taken every alternate visit and a prefilled requisition form was given with the instruction to get it done at nearby center having pathology laboratory facility if patient could not come at time of appearance of symptoms. At each visit the patients were evaluated for signs and symptoms of adverse events by trained staff and adherence to treatment schedule was confirmed for both the groups. Sputum examination was repeated at fourth week in all sputum AFB positive cases.

Hepatotoxicity gradation was based on the WHO classification and defined by liver enzyme level, however, aspartate transaminase (AST) level was preferred instead of alanine transaminase (ALT) level as it is well known that AST/ALT ratio > 2 is one important criteria for differential diagnosis between drug or chemical and viral hepatitis<sup>[33]</sup>. Grade I for any AST level of 51-125 U/L; grade II for any AST level of 126-250 U/L; Grade III for AST level of 251-500 U/L and grade IV for any level greater than 500 U/L or more than 250 U/L with symptoms of fulminant hepatitis. In case of grade I or II liver injury ATT was continued for 2 wk and enzymes were repeated. In grade III and IV

hepatitis ATT was stopped till enzyme level came to normal. AST was checked weekly, once its rise beyond 2 fold was confirmed after initial 2 wk follow-up till it came to normal level. Patients were called for monthly follow-up visits after completion of scheduled treatment for 3 mo in both the groups.

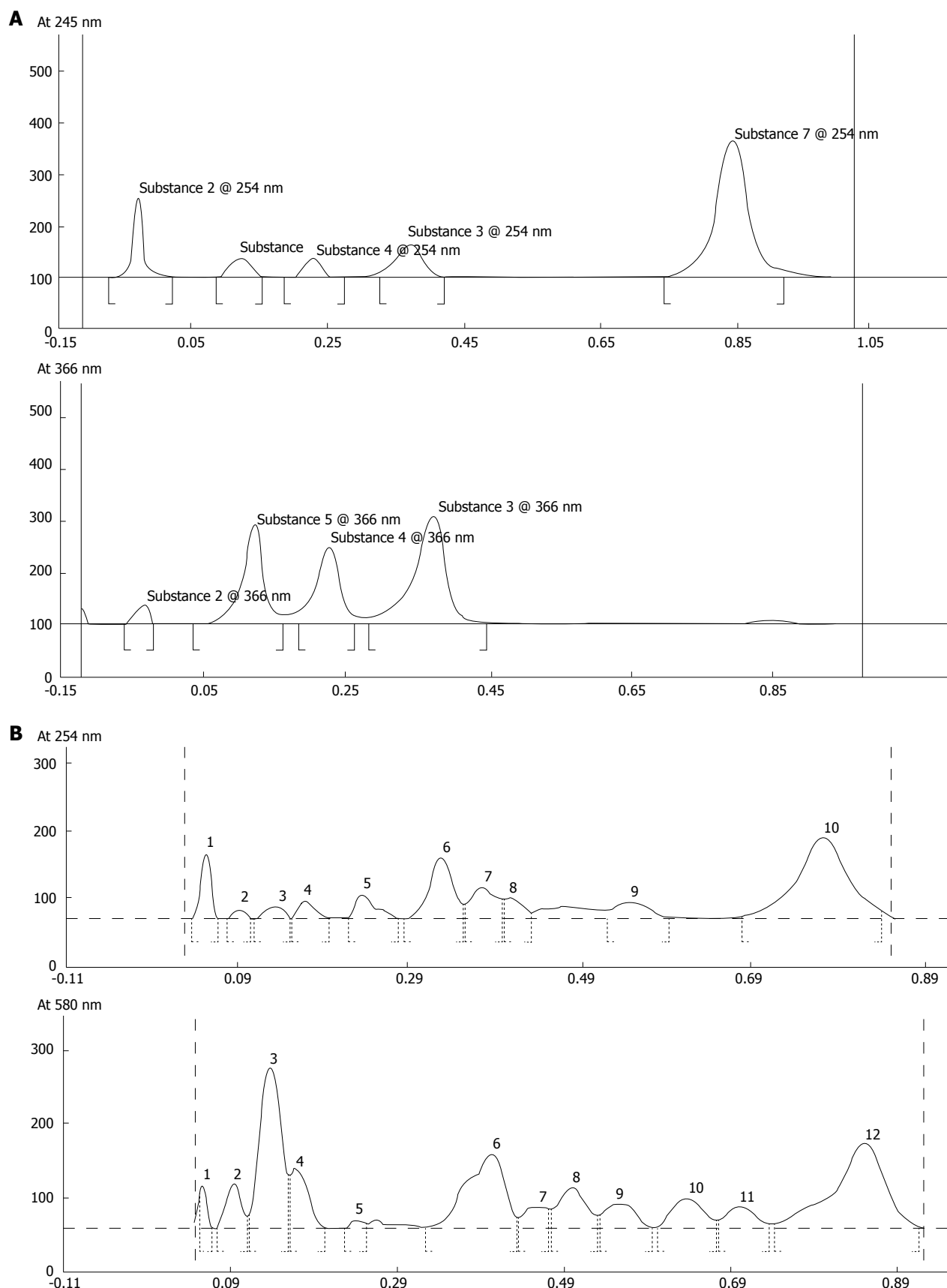
### Outcomes

Primary endpoints were to observe the development of hepatotoxicity in both the groups with assessment of severity by biochemical parameters and liver function tests if the hepatotoxicity exceeded Grade III parameters. Secondary outcome was to assess the impact of adjuvant medicine on the outcome of TB itself as defined by follow-up investigations, clinical cure and functional improvement<sup>[34]</sup> by the end of scheduled ATT and follow-up period. Completion of the regimen was defined as taking > 80% of scheduled medication for both the groups. Occasional breaks of one or two weeks due to some tribal festivals or an episodes of minor illness were not considered to be a break in the treatment.

### Statistical analysis

A sample of approximately 500 patients would provide 99% power in two sided tests with an  $\alpha$  value of 0.05 to detect a decrease in expected incidence of hepatotoxicity of any grade from 15% in control to < 3% in trial group.

Nominal and ordinal data in patient characteristics were described with percentage, median and inter



**Figure 2 A:** HPTLC finger print of formulation of *Curcuma longa*. Sample was extracted in MeOH and Solvent system for Mobile phase used was Chloroform: MeOH (99.5: 0.5). Note: Before loading the material on TLC plate, it was impregnated with di NaHPO<sub>4</sub> (anhydrous)-1.25 g dissolved in 25 mL double distilled water (DDW). **B:** HPTLC finger print of formulation of *Tinospora cordifolia*. Sample was extracted in-MeOH and NH<sub>4</sub>OH: MeOH (9:1) and solvent system for mobile phase used was -Chloroform: MeOH (8:2).

quartile range (IQR) between 25% and 75%, while measurement data by mean ± SD. Significance for each

characteristic was tested to ensure basic homogeneity of both the groups. Normality test was performed as the patients were representative of a tribal ethnicity and belonged to a disease that may have specific propensity for a subgroup, so the distribution may not be Gaussian despite a large sample. Those parameters failing the normality test were further analyzed by Wilcoxon's rank sum test and those passing it were tested by unpaired *T* test with Welch's modification where variance was unequal. Student's *T* test and  $\chi^2$  test with Yate's correction were done as needed.  $P \leq 0.05$  was considered significant. Number needed to treat (NNT) was decided for the adjuvant as test therapy. Finally more common outcomes including completion of treatment and response to treatment were analyzed by Fisher Exact test. Analyses were done by using KyPlot Version 2 beta 15, GraphPad InStat Version 3.06 and GraphPad StatMate 2 version 2.00.

## RESULTS

### Clinical and demographic characteristics at baseline

As shown in Figure 1, 528 patients were enrolled at six centers of SGSC mobile clinic. A high proportion of patients were of tribal ethnicity from Chaudhari, Gamit, Vasava and Bhil community known to carry sickle cell trait in up to 30% of population<sup>[35]</sup> and disease in 1.5%<sup>[36]</sup>, as reported by different governmental agencies. Eight patients from control and 12 patients from trial group were excluded from study due to violation of the protocol, hence control had 192 and trial had 316 patients for final analysis. As seen in Table 3 the median age and IQR for both the groups were quite similar. Also the two groups were similar in sex, body weight, habits, disease profile, baseline hematologic means and liver function except that trial group contained significantly higher number of patients with Cavitory Koch's than the control group.

### Hepatotoxicity

Tables 4 and 5 depict the effects of herbal adjuvant on primary outcome in the form of significantly decreased incidence of hepatotoxicity (2/316 *vs* 27/192) in the trial group as compared to control group. Mean onset of asymptomatic hepatitis was delayed in trial group and none in the trial group suffered from symptomatic hepatitis as against 63% of control group hepatitis patients. Grades II and III hepatotoxicity were observed in control group only. Serum AST, ALT and bilirubin level were raised in control group much significantly when compared with trial group hepatotoxicity cases.

### Disease outcome

As shown in Table 6, the number of sputum-positive cases after 1 mo of treatment in the trial group (2.9%) was significantly lower than control group (14.93%). Incidence of poorly resolved parenchymal lesion by roentgenogram and failure to improve functional status by ECOG score<sup>[34]</sup> being significantly lower and treatment dropouts nil in the trial group proved the efficacy of herbal adjuvant formulation.

**Table 3** Baseline demographic and disease characteristics in the intention-to-treat population

Characteristics of patients	Control	Trial	<i>P</i>
Sex			
Male (%)	104 (54)	169 (53)	0.934
Female (%)	88 (46)	147 (47)	0.927
Age-yr median(IQR 25th & 5th)	35 (23.75-45)	35 (27-45)	0.229
Weight (kg, mean $\pm$ SD)	37.61 $\pm$ 5.94	38.32 $\pm$ 6.08	0.199
Ethnicity (%) <sup>1</sup>			
Tribals	171(89)	287(91)	0.883
Others	21(11)	29(9)	0.559
Habits (%)			
Smoking	58 (30.2)	103 (32.6)	0.685
Alcohol	23 (12)	33 (10.4)	0.633
Both	26 (13.5)	53 (16.7)	0.403
Socio-Economic status (%)			
Upper-Middle	20 (10.4)	40 (12.6)	0.499
Middle	30 (15.6)	52 (16.4)	0.833
Lower	142 (74)	224 (71)	0.763
Pulmonary (%) <sup>2</sup>			
Open cases (sputum +ve)	67 (35)	137 (43.4)	0.214
Parenchymal	152 (79.2)	258 (81.6)	0.822
Cavitory	18 (9.4)	66 (21)	0.003
Effusion	9 (4.6)	20 (6.3)	0.464
Fibrosis	27 (14)	68 (21.5)	0.081
Extra pulmonary (%) <sup>2</sup>			
Cervical lymph node	18 (9.4)	31 (9.8)	0.883
Abdominal	4 (2.1)	6 (2)	0.886
Laryngeal	7 (3.6)	11 (3.5)	0.925
Central nervous system	6 (3.1)	6 (2)	0.389
Bones/Joints	10 (5.2)	19 (6)	0.72
Skin	0	3 (1)	0.598
Genito-Urinary	2 (1.04)	1 (0.3)	0.304
Type of case (%)			
New	140 (73)	195 (61.7)	0.245
Relapse	43 (22.4)	97 (30.7)	0.122
Chronic	9 (4.7)	24 (7.6)	0.225
Hematology			
Hb (%)	8.84 $\pm$ 1.93	8.95 $\pm$ 1.95	0.808
Total leucocyte count/mm <sup>3</sup>	10 179 $\pm$ 4 680	9575 $\pm$ 4075	0.478
ESR	66.79 $\pm$ 30.64	70.49 $\pm$ 27.91	0.517
Liver Function (mean, 95%CI)			
Serum bilirubin	1.07 (0.7-1.3)	1.09 (0.8-1.4)	0.952
AST	34.35 (17-63)	32.4 (11-61)	0.658
ALT	27.45 (9-57)	31.15 (6-52)	0.655
ALP	100 (50-260)	107 (50-260)	0.092

Unless stated otherwise, *P* values were calculated with the use of a two sided *t*-test with Welch's modification (when unequal variance) for continuous data and a  $\chi^2$  test for categorical data. <sup>1</sup>Ethnic group was assessed by investigation on screening. Tribal consists of Chaudhari, Gamit, Vasava, Bhil, Valvi, Nayka and Halpati. <sup>2</sup>Patients may belong to more than one category. ALP: Alkaline phosphatase.

### Weight and hematologic outcome

As seen in Table 3 and Table 6, the mean weight in control increased to 39.17  $\pm$  5.5 kg from 37.61  $\pm$  5.94 kg before ATT ( $P = 0.0063$ ) and in trial group mean weight was 40.65  $\pm$  6.22 kg from 38.32  $\pm$  6.08 kg before ATT and herb treatment ( $P < 0.0001$ ). While comparing the weight gain, trial group showed significantly higher weight gain than control group. Mean Hb of control and trial groups increased significantly after therapy; however, the inter group difference was nonsignificant. Erythrocyte sedimentation rate (ESR) in both groups decreased significantly compared from start to end of the treatment period; however, inter group comparison

**Table 4** Effects of herbal formulation on incidence, onset, duration and severity of hepatitis

ATT induced hepatitis outcome (Table 2)	Control (n = 192)	Trial (n = 316)	P
Patients with raised AST (%)	27 (14)	2 (0.63)	< 0.0001
Patients with raised S bilirubin (%)	17/27 (63)	0	< 0.0001
Mean onset of hepatitis symptomatic	49.12 ± 17.22	-	< 0.0001
Mean onset of hepatitis asymptomatic	29.9 ± 17.63	39 ± 11.31	0.443
Mean onset of any hepatitis	42 ± 19.48	39 ± 11.31	0.776
Mean duration of raised AST	27.5 ± 7.25	21	< 0.0001
Hepatitis gradation			
Grade-I	10	2	< 0.0001
Grade-II	9	0	< 0.0001
Grade-III	8	0	< 0.0001
Grade-IV	0	0	
Reinstitution of ATT in patients (%)	22/27 (81.5)	NA	

P calculated by  $\chi^2$  (categorical data) and two-sided Student's *t*-test with Welch's procedure (continuous data with unequal variance). NA: Not applicable.

**Table 5** Comparison of liver enzymes and bilirubin in patients developing hepatitis

	Control (n = 27)	Trial (n = 2)	P
AST	195.93 ± 108.74	85 ± 4.24	< 0.0001
ALT	75.74 ± 26.54	41 ± 1.41	< 0.0001
AST/ALT	2.35 ± 0.74	2.08 ± 0.18	0.207
ALP	241.74 ± 140.96	147.5 ± 45.96	0.118
Serum bilirubin	5.4 ± 3.4	1.5 ± 0.42	< 0.0001

Two-sided Student's *t*-test with Welch's procedure for continuous data with unequal variance. ALP: Alkaline phosphatase.

**Table 6** Effects of herbal formulation addition on disease outcome

Treatment outcome for ATT	Control	Trial	P
Number of sputum +ve case at start (%)	67/192 (35)	137/316 (43)	0.03
Number of sputum +ve case after 1-month of treatment (%)	10/67 (14.93)	4/137 (2.9)	0.0068
Poorly resolved parenchymal lesion (%)	9/152 (5.92)	2/258 (0.78)	0.0037
Failure to improve functional status (%)	19/192 (10)	6/316 (2)	0.00013
Treatment dropouts (%)	10/192 (5.2)	0	0.0001
Weight (kg)	39.17 ± 5.5	40.65 ± 6.22	0.0016
Hb (%)	10.92 ± 2.01	11.17 ± 1.97	0.176
ESR mm in 1st hour	45.96 ± 18.52	38.84 ± 22.37	0.0001

P calculated by  $\chi^2$  (categorical data) and two-sided Student's *t*-test with Welch's procedure (continuous data with unequal variance).

that was nonsignificant at the start showed a significantly more decrease in trial group at completion.

#### Non-hepatotoxic adverse events (data not shown)

Non-hepatotoxic adverse events occurred occasionally in both the groups and included nausea, vomiting, skin rash, epigastric pain and discomfort, malaise, dizziness,

arthralgia, peripheral neuropathy, anorexia and insomnia and sickle crisis. They were treated symptomatically with appropriate medications by the physician. Early dropouts occurred in both groups due to allergic reactions to ATT and they were excluded from the analysis as shown in Figure 1.

## DISCUSSION

The present clinical trial was based on unpublished results of previous preclinical studies at our center. As the conventional ATT has been well established and the Ayurvedic herbs have excellent safety profile as described previously, a phase I trial omission was granted. As the efficacy of the herbs was shown in concurrent administration design in an animal model of ATT induced hepatotoxicity<sup>[22]</sup>, it was considered scientific, as well as ethical, to try a similar model for a human clinical trial, hence herb treatment was started along with ATT at the same time and continued until completion of ATT.

Curcumin has anti-inflammatory, free radical scavenging and hepatoprotective activities tested *in vitro*. It suppresses production of superoxide by macrophages, exerts potent anti-inflammatory action that inhibits production of tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL) 1- $\beta$  and the activation of NF- $\kappa$ B in human monocytic derived cells<sup>[37,38]</sup>. Also, it has a strong antioxidant property and it inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates, by maintaining the activity of SOD, catalase and glutathione peroxidase at a higher level<sup>[39]</sup>. These properties clearly explain the hepatoprotective activity of CL in preclinical studies and present trial as well. Curcumin has antiviral, antiprotozoal, antibacterial, anti inflammatory, and antioxidant actions, in addition to its hepatoprotective activity, making it a suitable adjuvant agent that actually enhanced efficacy of ATT in present study.

In preclinical studies, TC has been shown to induce enzymes of drug metabolism and antioxidant system and to inhibit lipid peroxidation in mice. Cytochrome P450, NADP-Cytochrome (P sub 450) reductase, glutathione-s-transferase, DT diaphorase, SOD and catalase are enhanced. These effects improve liver function, protect against toxic assaults and increase protein synthesis by liver<sup>[40]</sup>. These activities can account for its hepatoprotective potential. It is a known immunostimulant enhancing cell mediated as well as humoral immune response in mice. Treatment of mice with dried stem crude extract prevented cyclophosphamide induced myelosuppression as well as immunosuppression<sup>[41]</sup>. Improved humoral immunity, enhanced macrophage and Kupffer cell function, enhanced neutrophil function, antioxidant activity and excellent hepatoprotective potential might explain the result of present clinical trial. Since many years the concept of classical phytotherapy using herbal drug combinations with superior efficacy and lesser side effects in comparison with single isolated constituents

of plant extracts has been repeatedly assessed clinically as well as pharmacologically. The exact mechanisms of action underlying these synergy effects are unknown. It could be explained by a multitarget action of compounds on a molecular level or partly by an improved resorption rate and a change of pharmacokinetics<sup>[42]</sup>. Hence, a formulation of CL and TC was used with an idea to enhance the potency due to synergistic action that was backed by promising results of a small preclinical pilot study (unpublished). As such, many standard Ayurvedic preparations also contain both of these herbs, albeit in a lower dose along with other multiple herbs.

Regarding the design of the trial, the strength lied in the base line characteristics which were remarkable similar and all the parameters for hepatotoxicity were objective and being tested by laboratory technicians at different places and totally unaware about the trial or outcome, so there could not be any chance for a bias in the detection and gradation of hepatotoxicity. In many complex clinical situations adaptive random allocation methods are implemented to maintain balance between stratification variables<sup>[43]</sup>; however, periodic evaluation revealed acceptably similar distribution among groups even with permuted block randomization. When one treatment or an intervention is expected to be superior; a Bayesian adaptive randomization (BAR) method is considered desirable to conventional randomization because it utilizes the accruing data in a “learn-as-you-go” fashion that arguably makes more sense than ignoring the trial’s data until it is completed<sup>[44]</sup>. BAR would allocate more numbers of patients to the potentially advantageous group without compromising the randomization, but making the trial design very complicated. Instead the control arm was truncated for ethical reasons with caution at periodic evaluations so that no dissimilarity in favor of the trial group should occur.

Though sickle cell disease patients were excluded from the study, persons having sickle cell traits are prone to have anemia, repeated chest infection and theoretically sickle cell crisis in extremes of situations with dehydration and acidosis. In the present trial two events of sickle cell crisis occurred in the control group. They might have phenotypic sickle cell disease<sup>[45]</sup>.

The average weight of the study population was around 38 kg in both the groups confirming malnutrition in addition to effect of TB alone. The significance of this characteristic could not be overemphasized because a low weight would culminate in to a higher per kg dosage of the hepatotoxic ATT. Considering the average weight to be 38 kg, the per kg dose of INH, RFM, PZA and EMB would come to 7.9, 11.84, 26.31 and 21 mg, respectively. Comparing this dose with a previous study<sup>[46]</sup> in which the per kg doses for INH, RFM and PZA were 5, 10 and 25-30 mg, respectively, and the period for observation was 59 d with reported hepatotoxicity to be 11%; it is apparent that the chances of developing hepatotoxicity would be definitely more for the patients in present study. In addition to malnutrition as a known risk factor, a higher per kg dose could also play some role.

With all these background, the results of present trial carried utmost importance that hepatotoxicity did not occur in the trial group despite a slightly unfavorable profile in the form of significantly higher number of patients with Cavitary pulmonary TB. Only two instances of raised AST were detected during monthly surveillance of liver enzymes and ATT was not stopped as it was asymptomatic grade I hepatotoxicity. Extensive disease, relapse cases, multiple relapse cases, miliary TB cases and cases with malnutrition, HIV carrier, and hepatitis B and C carrier, sickle cell trait, other back ground illnesses like COPD, hyper-eosinophilic syndrome, MB leprosy, diabetes mellitus, asthma, rheumatoid arthritis were all recruited in the control as well as the trial group and yet no incidence of clinically significant hepatotoxicity occurred in trial group, suggesting that regular administration of adjuvant herbs had taken care of all the known, hypothesized and unknown risk factors predisposing to ATT induced hepatotoxicity in clinical cases of TB.

Though with a power of 99% and highly significant out-come, this was a pilot study and much has remained to be done to exploit the full potential of the hepatoprotective ability of these herbs in cost-effective manner with defined recommendation for different subclasses of patients including latent TB cases and different high risk groups of clinical cases. A separate study with a low risk control, a high risk control and a high risk trial would serve both the purposes of testing the efficacy of herbs in high risk group as well as the validity of the hypothesis that low risk group has lower incidence of hepatotoxicity in the same population having similar ethnicity. The answer to the question whether low risk patients should be subjected to adjuvant medication or not, could also be answered from the results of study with such a design.

As latent TB cases on different preventive regimes have been shown to be at a greater risk for developing hepatotoxicity<sup>[7-9]</sup>, a separate well-designed trial for this class of patients could not be over-emphasized. The efficacy of these herbs may also be tested in patients showing liver enzyme increase detected by surveillance to decide whether ATT could be continued without risk of it to progress to higher grade hepatotoxicity as it is observed that sooner the detection, lesser the risk and later the appearance of hepatitis, greater the chances of fulminant failure<sup>[47]</sup>.

The NNT analysis of the study with a single outcome of hepatoprotection would come to eight. This is because the herbs were given to all as an adjuvant medicine where the possibility of developing hepatitis itself was expected to be between 11% and 15%. Looking to the result on disease profile it is clear that the sputum conversion to negativity was significantly superior in herb treated group despite an unfavorable sample, resolution of parenchymal lesion was significantly higher, failure to improve functional status was significantly lower and dropout rate came to nil. If these treatment factors are added then NNT would surely come lower.



Weight and hemoglobin both increased and ESR reduced significantly on intra group pre- and post-treatment comparison in both the groups proving the efficacy of conventional ATT alone too. The inter group comparison post treatment showed a significant advantage in weight gain and reduction in ESR, but not in hemoglobin level in trial group suggesting the superiority of ATT plus adjuvant herbs over ATT alone. It is apparent that iron and vitamin supplements were being given to anemic patients as per diagnosis and role of ATT must be supportive only in rising the Hb. Tumor necrosis factor alpha (TNF) has been shown to cause hypoferrremia and reduced intestinal iron absorption in mice and Curcumin inhibits TNF thereby having the potential to enhance iron absorption and help alleviate anemia<sup>[48,49]</sup>. Rise in Hb was not a direct function of both modalities and effects of curcumin on iron absorption in humans has not yet been tested; so the only valid comment would be that the theoretical advantage of CL was not observed practically in present study.

In conclusion CL and TC given as an adjuvant to standard ATT to any kind of TB patients prevented hepatotoxicity very significantly in terms of incidence, duration and severity and also helped improve outcome in terms of quicker and more efficient achievement of sputum negativity in open, potentially infectious cases; better response in parenchymal lesion resolution and helped improve patient compliance. Treated cases had better weight gain and significantly more reduction in ESR. A prospective trial for latent TB and MDR TB contacts can further exemplify its usefulness. Post-treatment design on detection of hepatotoxicity and concurrent administration design for different subgroups may clarify its efficacy in different clinical situations and provide a basis for evidence based recommendation for different sub groups of TB patients.

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## COMMENTS

### Background

Isoniazid, rifampicin and pyrazinamide, three major drugs of intensive phase of conventional anti-tuberculosis (TB) treatment (ATT), are hepatotoxic and the incidence varies between 4%-11%. Healthy contacts of TB patients (latent TB cases) are more prone to develop hepatotoxicity by presently recommended regimes. There is no established drug therapy to cure or prevent hepatotoxicity except stopping the treatment for a while and restoring it gradually at a later date when liver enzymes come to a normal level. Stopping the therapy may increase morbidity or prolong disability, while shifting to suboptimal non-hepatotoxic regime for time being poses the risk of emergence of resistant strains.

### Research frontiers

Search for non-toxic and highly effective new compounds for treating

tuberculosis or an effective vaccine conferring sustained protective immunity have largely failed. In literature there are many published preclinical studies showing herbs to prevent CCl<sub>4</sub> and paracetamol induced liver damage in rodents. Recently, four Ayurvedic herbs were shown to prevent ATT induced hepato-toxicity and immunosuppression in Guinea pigs. (<http://www.wjgnet.com/1007-9327/13/3199.asp>). But no clinical trial of hepatoprotective compound has ever been carried out in patients taking conventional ATT.

### Innovations and breakthroughs

The present study is the first of its kind and the results are encouraging for the prevention of hepato-toxicity and improving the disease outcome by greater sputum clearance in Cavitory lesions and better resolution of parenchymal lesions. Thus, addition of two simple herbs makes the conventional ATT much safer and efficacious.

### Applications

Incorporation of such a formulation in current conventional ATT at a mass level may contribute a lot to minimize the risk of liver injury, improve patient compliance and disease outcome that might ultimately help to control emergence of MDR strains of AFB. If similar protection is observed in latent tuberculosis by a prospective trial, it would be a boon for the latent TB cases subjected to a serious risk by preventive ATT.

### Peer review

The manuscript is well written. This is an interesting trial. The study is nicely designed and the analytical data appear to be technically and scientifically sound. The outcome is remarkable and clearly shows beneficial effect of the herbal formulation in hepatic injury by ATT.

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