Liv.52 in the Prevention of Hepatotoxicity in Patients Receiving Antitubercular Drugs: A Meta-analysis

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ABSTRACT

The aim of the study was to conduct a meta-analysis on the efficacy and short- and long-term safety of Liv.52 as a hepatoprotective in tuberculosis (TB) patients receiving anti-TB drugs, as published in eight controlled clinical trials. A meta-analysis of eight clinical studies conducted between 1970 and 1992 in 689 tubercular patients receiving antitubercular treatment (ATT) along with Liv.52 or placebo was taken up for this study. Duration of the treatment varied from four weeks to one year. Children below the age of two years received 10-20 drops of Liv.52 three times daily. Children in the age group of 2-5 years received 20 drops of Liv.52 three times daily. Children aged >5 years and adults received 1-2 teaspoonsful of Liv.52 syrup three times daily or 1-2 tablets of Liv.52 two to three times daily. Improvement in the various parameters of hepatotoxicity, such as hepatomegaly, anorexia, weight gain, general well-being and liver function test (ALT), and improvement in the ultrasonographic findings of the hepatobiliary system were taken into consideration. Changes in various parameters from baseline values and those at the end of the study were pooled and analyzed cumulatively using Fischer’s exact test or unpaired Students’ ‘t’ test. Statistical analysis was performed using GraphPad Prism Software (Version 4.03). Results of the meta-analysis showed a statistically significant improvement in hepatotoxicity in patients receiving anti-TB drugs and Liv.52. Significant improvements were observed in associated symptoms such as anorexia, weight gain, hepatomegaly and general well-being. The protective effect of Liv.52 against hepatotoxic reaction caused by ATT was further substantiated by a significant reduction in alanine aminotransferase (ALT) values and alleviation of gastrointestinal symptoms due to hepatitis. No adverse effects were reported or observed due to Liv.52 during the study period and the compliance to the drug therapy was good. Therefore, from the above findings it can be concluded that Liv.52 acts as a hepatoprotective in the hepatotoxicity of tuberculosis patients receiving ATT.

Key words: Meta-analysis, Liv.52, hepatotoxicity, anti-TB drugs

Tuberculosis (TB) is a common problem worldwide, especially after the recent increase in the incidence of acquired immunodeficiency syndrome (AIDS) and multiple drug resistant tuberculosis (MDR-TB) due to inefficient management.1 Every year, an estimated eight million new cases and two million deaths occur worldwide due to TB.2 At present, most commonly used anti-TB drugs are more or less hepatotoxic, especially when several of them are used in combination. Liver dysfunction caused by anti-TB drugs often results in interruption of antitubercular treatment (ATT) and acute hepatic failure, which is life-threatening.3,4 Drug-induced hepatotoxicity is a potentially serious adverse effect of anti-TB treatment regimens containing isoniazid, rifampicin and pyrazinamide.5

The underlying mechanism of ATT-induced hepatotoxicity and the factors predisposing to its development are not clearly understood. Age and sex of the patients, chronic alcoholism and chronic liver disease, hepatitis B virus carrier status and acetylator and nutritional status have all been incriminated as possible predisposing factors in earlier studies. As enzymes for drug metabolism in hepatocyte microsomes may have congenital defects, malformation and low activity or may be inhibited by drugs; drugs or drug metabolites are very toxic to hepatocytes. The other reason is hypersensitivity to drugs. The drugs as a hapten cause allergic reaction by immune mechanism, leading to an increase in alanine aminotransferase (ALT) alone in clinical situation.6

Commonly used anti-TB drugs, such as isoniazid, rifampicin and pyrazinamide, are hepatotoxic.6 Isoniazid causes hepatic damage by either toxicity
or hypersensitivity induced by its metabolite-acetylhydrazine. Rifampicin may accelerate the metabolism of isoniazid as a strong enzyme inducer, resulting in an increase in acetylhydrazine. This combines with biomacromolecules in liver, leading to hepatocellular damage (usually seen in aged patients with excessive drinking) malnutrition, or liver ailment. Pyrazinamide hepatotoxicity is dose-dependent and the general dose rarely causes hepatic damage. Isoniazid and rifampicin are the first-line anti-TB medicines because of their strong bactericidal effects.\(^7,8\)

The clinical presentation of ATT-associated hepatitis is similar to that of acute viral hepatitis. ATT can cause varied degree of hepatotoxicity from a transitory asymptomatic rise in transaminases to acute liver failure. The frequency of hepatotoxicity in different countries varies widely from 2% to 39%.\(^9\) The occurrence of drug-induced hepatotoxicity is unpredictable, but it is observed that certain patients are at a relatively higher risk as compared to others.

Liv.52, a polyherbal formulation, has been used extensively in the management of various hepatic disorders over the past 55 years. The principal herbs used in the preparation of Liv.52 include *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Terminalia arjuna*, *Cassia occidentalis*, *Achillea millefolium* and *Tamarix gallica*.

Liv.52 was evaluated for its efficacy in the prevention of hepatotoxicity induced by anti-TB drugs in 689 TB patients receiving ATT, as reported in eight controlled clinical trials conducted between 1970 and 1992. Results of each clinical trial with Liv.52 showed significant hepatoprotective effect, both clinically and as determined through liver function test (ALT). The study also showed good short- and long-term safety. These studies need to be meta-analyzed to further substantiate the clinical efficacy of Liv.52 as a hepatoprotective in patients with ATT.

A meta-analysis is a two-stage process; the first stage is the extraction of data from each study and the calculation of result for each study. The second stage involves deciding whether it is appropriate to calculate a pooled average result across studies. This process gives greater weightage to the results from the studies that give more information.\(^10\) Advantages of meta-analysis include deriving and generalization of the population studies, ability to control between study variation, and statistical testing of overall factors/effect size parameters in related studies.

To cumulate the result of all the studies, a meta-analysis was done to analyze the efficacy and short- and long-term safety of Liv.52 as a hepatoprotective in patients with TB receiving ATT.

**Aim of the Study**

The aim of the study was to evaluate the efficacy and short- and long-term safety of Liv.52 in patients receiving ATT, as reported in eight controlled clinical trials.

**Material and Methods**

**Study Design**

This is a cumulative meta-analysis of eight published controlled clinical trials of Liv.52 in the prevention of hepatotoxicity in patients receiving anti-TB drugs. Of these eight controlled trials, one was a double-blind placebo-controlled study and the remaining seven were controlled studies.

**Inclusion Criteria**

All published studies, which evaluated the efficacy and safety of Liv.52 in hepatotoxicity due to ATT, were included in the meta-analysis irrespective of the study design. The meta-analysis included eight controlled clinical trials and there were no restrictions regarding sex, age or duration of the disease. The outcome variables included measurement of data on changes in clinical symptoms and signs of hepatotoxicity, laboratory results, and incidence of adverse events during/after the treatment.

**Exclusion Criteria**

Phase I studies conducted with Liv.52 and uncontrolled studies were excluded from the analysis.

**Study Procedures**

Eight controlled clinical studies conducted between 1970 and 1992 in 689 patients with TB receiving ATT and presenting with hepatotoxicity were subjected to meta-analysis. Duration of the treatment varied from four weeks to one year. Children below the age of two years received 10-20 drops of Liv.52 3-4 times daily. Children in the age group of 2-5 years received 20 drops of Liv.52 three times daily. Children aged >5 years and adults received...
1-2 teaspoonsful of Liv.52 syrup three times daily or 1-2 tablets of Liv.52 two to three times daily. Improvement in the various parameters of hepatotoxicity, such as hepatomegaly, anorexia, weight gain and general well-being, and improvement in the ultrasonographic findings of the hepatobiliary system were taken into consideration. Changes in various parameters from baseline and at the end of the study were pooled and analyzed.

The incidence and type of adverse events reported by various studies were also tabulated separately. All adverse events, either reported or observed by patients or investigators, were recorded with information about severity, duration and action taken regarding the study drug. Relation of adverse events to study medication was predefine as 'Unrelated' (a reaction that does not follow a reasonable temporal sequence from the administration of the drug), 'Possible' (follows a known response pattern to the suspected drug, but could have been produced by the patient’s clinical state or other modes of therapy administered to the patient), ‘Probable’ (follows a known response pattern to the suspected drug that could not be reasonably explained by the known characteristics of the patient’s clinical state), and ‘Certain’ (the adverse events must have definitive relationship to the study drug, which cannot be explained by concurrent disease or any other agent).

Primary and Secondary Outcome Measures
Primary predefined outcomes were clinical recovery from hepatotoxicity due to anti-TB drugs. Secondary outcomes were safety and compliance to Liv.52.

Statistical Analysis
Values are expressed as incidences of patients with or without symptoms or mean ± SD. Changes in various parameters from baseline values and values at the end of the study were pooled and analyzed cumulatively using Fischer’s exact test or unpaired Students’ ‘t’ test. The minimum level of significance was fixed at 95% confidence limit and a two-sided p < 0.05 was considered significant. Statistical analysis was performed using GraphPad Prism Software, Version 4.03 for windows (GraphPad Software, San Diego, California, United States).

Results
The demographic details of the clinical trials are provided in Table 1. Among the eight controlled studies, one was a double-blind placebo-controlled study. A total of 689 patients with TB receiving ATT presenting with hepatotoxicity were included in the study; of which 367 received Liv.52; 20 received placebo and 302 served as controls. The age varied from 2-month-old infant to 60 years and the duration of the treatment varied from 4 weeks to one year (Table 1). Details of the eight controlled clinical trials are enumerated in Table 2.11-18

Among the 220 patients presenting with anorexia, who received Liv.52 in addition to ATT, only 16 patients had anorexia at the end of the treatment, showing a significant improvement (p < 0.0001). Whereas in 163 patients (controls) who received ATT, anorexia was persistent in 160 (Table 3).

Among the 186 patients presenting with weight loss, who received Liv.52 in addition to ATT, only 23 did not gain weight at the end of the treatment, showing
a significant improvement (p < 0.0001). In the control group, of the 190 patients, 178 did not show any gain in the weight (Table 4).

Hepatomegaly was evaluated through clinical examination, symptom evaluation, and ultrasonographic examination. In patients treated with Liv.52, it was seen that out of 25 patients, 24 improved with regression of liver size as well as with the symptomatic improvement in the pain in the right hypochondriac region, showing a statistical significance of p < 0.0001, whereas in the control group, there was no improvement in any patient (Table 5).

Evaluation of general well-being in patients receiving ATT showed that of the 25 cases treated with Liv.52, 21 showed significant improvement (p < 0.0001). Whereas in control group, none of the cases showed any improvement (Table 6).

Among the 82 patients receiving ATT along with Liv.52, only one patient showed an increase in the ALT levels as compared to 15 cases out of 82 presenting with increased ALT levels following treatment with anti-TB drugs alone. The protection offered by Liv.52 in preventing the increased levels of ALT in patients received ATT was significant at p < 0.0003 (Table 7).

Among the 82 patients presenting with gastrointestinal symptoms and symptoms related to hepatitis, addition of Liv.52 showed a significant improvement (p < 0.0001) in patients receiving ATT. Whereas among the 82 patients receiving ATT as controls, 44 patients still presented with symptoms (Table 8).

In one clinical trial, the drug-drug interactions of rifampicin with Liv.52 was studied, which compared the initial and subsequent serum rifampicin levels in both control (ATT alone) and treated (Liv.52 + ATT) groups. The serum rifampicin levels in group 1 (control) and group 2 (Liv.52), on days 1, 15, and 30 were comparable and not significant, though there was a falling trend in the levels as compared to the initial and subsequent readings. It appears that the process of enzymatic induction witnessed with rifampicin metabolism is not influenced by the addition of Liv.52 (Table 9).

There were no adverse effects observed or reported during the clinical trials in patients who received Liv.52 along with ATT and compliance to the use of formulation was good. There were no drop outs or withdrawal from the study.

### Table 3. Effect of Liv.52 on Anorexia with ATT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Patients showing symptoms at the end of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>163</td>
<td>160</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>220</td>
<td>16*</td>
</tr>
</tbody>
</table>

Statistical analysis: Fischer’s exact test; *p < 0.0001 as compared to ATT alone.

### Table 4. Effect of Liv.52 on Weight Gain in Patients Receiving ATT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Patients showing no weight gain after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>190</td>
<td>178</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>186</td>
<td>23*</td>
</tr>
</tbody>
</table>

Statistical analysis: Fischer’s exact test; *p < 0.0001 as compared to ATT alone.

### Table 5. Protective Effect of Liv.52 on Hepatomegaly in Patients Receiving ATT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Regression of liver size after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>25</td>
<td>24*</td>
</tr>
</tbody>
</table>

Statistical analysis: Fischer’s exact test; *p < 0.0001 as compared to ATT alone.

### Table 6. Effect of Liv.52 on General Well-being in Patients Receiving ATT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>No. of patients showing improvement after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>25</td>
<td>21*</td>
</tr>
</tbody>
</table>

Statistical analysis: Fischer’s exact test; *p < 0.0001 as compared to ATT alone.

### Table 7. Protective Effect of Liv.52 on ALT Levels in Patients Receiving ATT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>No. of patients with increase in ALT levels after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>82</td>
<td>15</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>82</td>
<td>1*</td>
</tr>
</tbody>
</table>

Initial values of ALT <40 U
Statistical analysis: Fischer’s exact test; *p < 0.0003 as compared to ATT alone.

### Table 8. Protective Effect of Liv.52 on Protection of GI Symptoms and Symptoms Related to Hepatitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Patients showing symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATT</td>
<td>82</td>
<td>44</td>
</tr>
<tr>
<td>ATT + Liv.52</td>
<td>82</td>
<td>3*</td>
</tr>
</tbody>
</table>

Statistical analysis: Fischer’s exact test; *p < 0.0001 as compared to ATT alone.

### Table 9. Initial and Subsequent Serum Rifampicin Levels in Both the Groups after 2 Hours of Rifampicin Ingestion (in µg/ml)

<table>
<thead>
<tr>
<th>Day of estimation</th>
<th>Group 1 (control)</th>
<th>Group 2 (Liv.52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>8.5 ± 4.2</td>
<td>9.01 ± 5.3</td>
</tr>
<tr>
<td>15th day</td>
<td>7.6 ± 4.9</td>
<td>7.4 ± 4.7</td>
</tr>
<tr>
<td>30th day</td>
<td>7.1 ± 5.1</td>
<td>7.9 ± 4.5</td>
</tr>
</tbody>
</table>

Statistical analysis: Unpaired Students’ ‘t’ test.
Discussion

A useful definition of meta-analysis was given by Huque as "A statistical analysis that combines or integrates the results of several independent clinical trials considered by the analyst to be combinable."19

A single study often cannot detect or exclude with certainty clinically relevant differences in the effects of two treatments. Cumulative meta-analysis is defined as the repeated performance of meta-analysis whenever a new trial becomes available for inclusion. Such cumulative meta-analysis can retrospectively identify the point in time when a treatment effect first reaches conventional levels of significance.20

Meta-analysis thus not only consists of the combination of data but also includes the epidemiological exploration and evaluation of results (epidemiology of results).21 Therefore, new hypotheses that were not posed in single studies can be tested in meta-analyses.22 The number of patients included in clinical trials is often inadequate, as in some cases the required sample size may be difficult to achieve.23 Meta-analysis may, nevertheless, lead to the identification of the most promising or urgent research question and can permit a more accurate calculation of the sample sizes needed in future studies.24 Goals of meta-analysis are to enable the overall significance of an effect to be evaluated, based on the multiple studies available, and to estimate an overall effect size by combining the individual estimates in multiple studies.25

In the present meta-analysis, clinical trials and their details were tabulated and analyzed statistically. The clinical trials included in the meta-analysis consist of patients suffering from various types of TB such as pulmonary, abdominal, childhood TB, tuberculous meningitis, miliary TB and tubercular cervical lymphadenitis. The outcome of this analysis showed marked improvement with Liv.52 as a hepatoprotective in patients treated with anti-TB drugs, as indicated by relief in clinical symptoms, maintenance of ALT levels and regression of liver size (as observed clinically and ultrasonographically). The efficacy of Liv.52 is attributed to the potent hepatoprotective herbs present in the formulation, which is described below in detail.

C. spinosa was found to possess potent hepatoprotective activity against CCl₄-, paracetamol (in vivo)-, thioacetamide- and galactosamine (in vitro)-induced hepatotoxicity.26 Strong anti-inflammatory activity of C. spinosa was demonstrated, which was comparable to oxyphenbutazone.27 C. spinosa was found to possess significant antioxidant, antimicrobial and antifungal activities.28-30

C. intybus revealed hepatoprotective effect against CCl₄-induced hepatotoxicity as indicated by significant prevention of the elevation of malondialdehyde formation (plasma and hepatic) and enzyme levels (aspartate aminotransferase [AST] and ALT) along with restoration of the histoarchitecture.31,32 C. intybus showed significant increase in the number of circulating leukocytes, the weights of concerned organs (liver, spleen and thymus), number of splenic plaque-forming cells, hemagglutination titers and the secondary IgG antibody response against ethanol-induced toxicity. There were also significant increases in delayed-type hypersensitivity reaction, phagocytic activity, natural killer cell activity, cell proliferation and interferon gamma-secretion.33 Reports suggest that the observed hepatoprotective effect of C. intybus might be due to its ability to suppress the oxidative degradation of DNA in the tissue debris34 and potent antioxidant activity (radical scavenging effects, inhibition of hydrogen peroxide and iron chelation).35

It also showed marked cytoprotective activity, which was established against ethanol-induced liver damage. The cytoprotective activity was further supported by restoration of histoarchitecture of the liver.36

S. nigrum investigated against CCl₄-induced hepatic damage showed remarkable hepatoprotective activity as confirmed by evaluated biochemical parameters including AST, ALT, ALP and total bilirubin levels.37 It was also demonstrated to protect DNA against oxidative damage and suppress the oxidative degradation of DNA in the tissue debris.38 S. nigrum was found to possess a potent antioxidant activity, which was demonstrated by scavenging of hydroxyl radicals and DPPH radicals.39

The potent antioxidant activity of T. arjuna might be due to its effects on lipid peroxidation.40 T. arjuna was found to inhibit nitric oxide (NO) production41 and decrease inducible nitric oxide synthase (iNOS) levels in lipopolysaccharide-stimulated peritoneal macrophages.42 Potent antiviral (by virtue of inhibition of viral attachment and penetration) and antibacterial activities of T. arjuna were reported.43,44

Significant hepatoprotective effects of C. occidentalis in chemically induced liver damage were noted.45 C. occidentalis modulates hepatic enzymes and
provides hepatoprotection against cyclophosphamide-induced immunosuppression. Antimicrobial properties of \emph{C. occidentalis} were comparable with standard reference antibiotics. \emph{C. occidentalis} against \emph{Salmonella typhi} was demonstrated. Clinically beneficial effects of \emph{A. millefolium} in the treatment of chronic hepatitis was demonstrated. Similar clinical improvements in chronic hepatoclecytis and angiocholitis with \emph{A. millefolium} were established. Antioxidant and antimicrobial activities of \emph{A. millefolium} were also reported.

Therefore, as discussed above, these synergistic actions (hepatoprotective, antimicrobial, antioxidant and anti-inflammatory) exhibited by the ingredients of Liv.52 might provide the protective action against hepatotoxic reaction in patients receiving ATT.

\section*{Conclusion}

This meta-analysis of eight controlled clinical trials revealed a highly significant hepatoprotective activity of Liv.52 in patients with TB receiving ATT, as evidenced from improvement in clinical parameters and liver function test (ALT). These remarkable results might be due to the synergistic activities of the potent hepatoprotective individual herbs of Liv.52. It was also observed that Liv.52 offered hepatoprotective activity without interfering with the pharmacokinetics of anti-TB drugs such as rifampicin, which is the most common first-line drug in ATT. There were no clinically significant adverse reactions due to Liv.52 in any of these studies. In all these eight studies, overall compliance to Liv.52 treatment among patients who received ATT was good, and no treatment discontinuations were reported. Therefore, it can be concluded from the meta-analysis that Liv.52 treatment prevented hepatotoxic reactions in patients who received ATT. These findings clearly suggest the beneficial role of Liv.52 in the management of drug-induced (ATT) hepatotoxicity.

\section*{References}