SHORT COMMUNICATION

Ameliorating effects of *Tamarindus indica* fruit extract on anti-tubercular drugs induced liver toxicity in rats

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The study aimed to evaluate the hepatoprotective potential of aqueous extract of *Tamarindus indica* fruit against combination of two antitubercular drugs viz. Isoniazid (INH) and Rifampicin induced hepatotoxicity in rats. In vitro antioxidant activity of aqueous extract of *T. indica* by DPPH–HPLC method was found to be 81.48%. Treatment with aqueous extract of *T. indica* significantly reduced the elevated levels of biochemical markers such as SGOT, SGPT, ALP, bilirubin, TBARS and increased the albumin level as well antioxidant activities of SOD, CAT and GSH in intoxicated rats. The biochemical changes were supported by histological observations. Results of this study clearly demonstrate that aqueous extract of *T. indica* fruit protects against anti tuberculosis induced oxidative liver damage in rats and thus possess significant hepatoprotective activity. Further, it could be suggested that supplementation with this food extract might prove beneficial in the individuals on anti-TB drugs.

**Keywords:** DPPH–HPLC; hepatoprotective; *Tamarindus indica*; tuberculosis

1. Introduction

Tuberculosis (TB) is an infectious bacterial disease caused by various strains of *Mycobacterium tuberculosis* (Kumar et al. 2007). Tuberculosis is a very common disease worldwide but its prevalence is quite higher in developing countries (Sharma 2004). Isoniazid (INH), rifampicin (RIF), pyrazamide (PZA), ethambutol, streptomycin etc., are the most commonly used drugs for
the treatment of TB (Ernst et al. 2007). INH and RIF belong to the first line anti-TB drugs and are known to cause hepatotoxicity (Awodele et al. 2010). The frequency and magnitude of liver toxicity almost get doubled when these two anti TB medications are co-administered (Trevor et al. 2004). These drugs produce liver injury by damaging liver membrane that leads to the leakage of bilirubin and other biochemical marker enzymes such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) etc. (Tasduq et al. 2005). There is a necessity to search, explore alternatives and develop new, safe, effective and affordable anti-TB drugs to overcome the side effects of the currently available anti-TB therapy (Gautam et al. 2007).

*Tamarindus indica* Linn., is a large, evergreen tree, commonly known as Tamarind in English belongs to the family of Leguminosae. Phytochemical investigation of tamarind indicated the presence of various biologically active secondary metabolites such as flavonoid glycosides, vitexin, orientin, homoorientin and hordenine (Prajapati et al. 2006). It is also rich in minerals and various phenolic compounds which makes it an important food. Tamarind fruit is also used as folkloric medicine to treat digestive disorders, constipation, cough and as blood tonic (Martinello et al. 2006). Other parts of this plant are also reported to exhibit significant biological actions (Tsuda et al. 1994; Rimbau et al., 1999; Martinello et al. 2006). To the best of our knowledge, no scientific study has been done to evaluate the potential of *T. indica* as a hepatoprotectant in INH and RIF induced hepatotoxicity. Therefore, considering the high incidence of tuberculosis as well as hepatitis in developing countries and insufficiency of any cheap and effective modern drug protecting liver, the present study aimed to investigate the hepatoprotective activity of aqueous extract of *T. indica* in INH and RIF induced hepatotoxicity in Wistar Albino rats by assessing biochemical parameters and histopathological studies.

2. Results and discussion

2.1. *In vitro DPPH radical scavenging activity by RP-HPLC*

In this study, *in vitro* HPLC–DPPH assay method was used to investigate the antioxidant activity of aqueous fruit extract of *T. indica* (AFTI). It was observed that AFTI at concentration of (1000 µg/mL) had radical scavenging activity of 81.48 ± 5.31% which was comparable to that of the standard ascorbic acid (100 µg/mL) (95.11 ± 7.89%) (Figure S1). The antioxidant activity of the plant extract could be attributed to the presence of flavonoid, ascorbic acid, poly phenol and B-carotene (Shammi et al. 2013).

2.2. *Acute toxicity study*

The plant extract was devoid of any toxicity as it did not cause any significant behavioural changes and also no mortality was observed at dose up to 3000 mg/kg. Hence, based on the acute toxicity results, two different dose levels of AFTI were selected for further study.

2.3. *Effect of the Tamarindus indica extract on serum biomarkers of liver toxicity*

The potential of tamarind to guard against the combined anti-TB drugs induced hepatotoxicity was investigated in rats. Administration of a single combined dose of INH (50 mg/kg i.p.) + RIF (100 mg/kg i.p.) anti-TB drugs lead to hepatic damage as evident by the significant elevation (*p < 0.01*) of serum levels of SGOT, SGPT, ALP and bilirubin in all groups as compared to normal control group I. The toxic control group treated with only anti-TB drugs showed an approximately 2.21-, 2.97-, 3.47- and 3.83-folds increase in the levels of SGOT (113.56 ± 6.61), SGPT (133.15 ± 8.07), total bilirubin (1.77 ± 0.10) and ALP (110.03 ± 12.27), respectively, as compared to normal control group SGOT (51.21 ± 6.85),
SGPT (44.87 ± 5.17), total bilirubin (0.51 ± 0.05) and ALP (28.70 ± 6.92). The increased concentration of these biomarkers is due to leakage from hepatic cells in the blood stream which indicate liver injury (Lee 2003). However, treatment with AFTI at 500 mg/kg p.o. for fourteen days decreased the levels of above mentioned parameters significantly (p < 0.01) and brought down the levels of SGOT (65.69 ± 7.18), SGPT (69.04 ± 6.51), total bilirubin (0.96 ± 0.11) and ALP (65.89 ± 6.16) to the normalcy, indicating reversal of the liver damage due to protective action. At lower dose (250 mg/kg) moderate hepatoprotective activity was observed w.r.t. normal control group but the reduction in the level of biomarkers was quite significant (p < 0.05) as compared to toxic control group II. Overall, the hepatoprotective actions of AFTI at 500 mg/kg dose level were found to be at par with the positive control, silymarin. Administration of single dose of INH and RIF also significantly decreased the level of serum albumin in groups II–IV, which was retrieved to normalcy in AFTI and standard treatment groups (Table 1). The reduction in the levels of biomarkers of hepatotoxicity by AFTI and reference drug could be due to the protection of hepatocytes membrane integrity that further prevents the leakage of serum makers in the blood circulation.

2.4. Effects of the Tamarindus indica extract on lipid peroxidation, cell glutathione and liver antioxidant enzymes

Administration of combined anti-TB drugs, (INH + RIF) caused an elevation in TBARS level with significant decline (p < 0.01) in GSH, SOD and CAT levels in homogenate tissues of all groups as compared to normal control group indicating depletion in intracellular antioxidant defence mechanism due to production and accumulation of lipid peroxides (Sasidharan et al. 2014). Treatment with AFTI at a dose of 500 mg/kg significantly reduced the elevated TBARS level (0.45 fold) and also raised the GSH, SOD and CAT levels (approximately 1.98, 2.05 and 2.51 folds, respectively) when compared with the toxic control group. The results indicate that AFTI has the ability to restore the activity of hepatic enzymes in anti-TB drugs induced hepatotoxicity. AFTI at a lower dose did not show very promising results as it reversed the levels of GSH, SOD and CAT approximately 1.13-, 1.05- and 1.18-folds, respectively. The results of this study demonstrate that the tamarind extract possesses ameliorative hepatoprotective effect and could have useful therapeutic implications in the TB patients who are on INH and RIF therapy (Table 2).

2.5. Histopathological studies

The histopathology examinations of the group I (normal control) animal liver tissues showed a normal lobular architecture of the hepatocytes along with a portal triad with normal structures. In contrast, the liver sections of the toxic group II showed marked necrosis and inflammation in the centrilobular region with portal triad confirming the liver damage. The histological architecture of liver sections of animal treated with AFTI (250 mg/kg) group showed the portal triad with moderate degree of inflammatory cell infiltration around the bile duct and animals treated with AFTI at 500 mg/kg revealed the centrizonal area with less degree of inflammatory cell infiltration. The results of histopathological studies were comparable with standard drug, silymarin and thus support the protective action of tamarind extract in drug induced hepatotoxicity (Figure S2).

3. Conclusion

It could be concluded that AFTI at higher doses possess significant hepatoprotective activity against anti TB induced oxidative liver damage in rats and, therefore, dietary supplementation of
Table 1. Effects of aqueous extract of *Tamarindus indica* on biochemical parameters in serum of the rats intoxicated with anti-tubercular drugs (anti-TB).

| Groups                               | Parameters               | SGOT (IU/L) | SGPT (IU/L) | Albumin (g/dl) | Total bilirubin (mg/dl) | ALP (KA unit) |
|--------------------------------------|--------------------------|-------------|-------------|----------------|-------------------------|---------------|
| Normal Control (I)                   |                          | 51.21 ± 6.85| 44.87 ± 5.17| 4.69 ± 0.07    | 0.51 ± 0.05             | 28.70 ± 6.92  |
| Toxic Control (anti-TB) (II)         |                          | 113.56 ± 6.61| 133.15 ± 8.07| 2.82 ± 0.06    | 1.77 ± 0.10             | 110.03 ± 12.27|
| Positive Control (Silymarin) (III)   |                          | 58.20 ± 5.24| 49.07 ± 7.88 | 4.26 ± 0.07    | 0.62 ± 0.06             | 33.40 ± 6.78  |
| *T. indica* (250 mg/kg) + (anti-TB) (IV) |                        | 100.08 ± 9.11| 127.08 ± 7.01| 2.91 ± 0.08    | 1.72 ± 0.07             | 95.7 ± 9.09   |
| *T. indica* (500 mg/kg) + (anti-TB) (V) |                        | 65.69 ± 7.18| 69.04 ± 6.51 | 3.73 ± 0.07    | 0.96 ± 0.11             | 65.89 ± 6.16  |

Notes: Each value is represented as mean ± SEM, No. of animals (n) = 6, *p < 0.01 when toxic control compared with control, ns = non Significance *p < 0.05, **p < 0.01 versus toxic control, One way ANOVA followed by Dunett’s Test.

Table 2. Effects of aqueous extract of *Tamarindus indica* on biochemical parameters in hepatic tissue of the rats intoxicated with anti-tubercular drugs.

| Groups                               | Parameters               | TBARS (nanomole of MDA/mg protein) | GSH (μmole GSH/mg protein) | SOD (nanomoles of H₂O₂ consumed/min/mg protein) | Catalase (U/mg protein) |
|--------------------------------------|--------------------------|-----------------------------------|---------------------------|------------------------------------------------|-------------------------|
| Normal control (I)                   |                          | 0.081 ± 0.011                     | 0.727 ± 0.12              | 4.45 ± 0.89                             | 9.05 ± 1.35             |
| Toxic control (anti-TB) (II)         |                          | 0.22 ± 0.05                       | 0.327 ± 0.05              | 1.76 ± 0.06                             | 2.33 ± 0.31             |
| Positive control (Silymarin) (III)   |                          | 0.086 ± 0.015**                   | 0.690 ± 0.08**            | 4.18 ± 0.41**                            | 8.02 ± 1.02**           |
| *T. indica* (250 mg/kg) + (anti-TB) (IV) |                        | 0.18 ± 0.07*                      | 0.371 ± 0.08**            | 1.84 ± 0.08*                            | 2.75 ± 0.09*            |
| *T. indica* (500 mg/kg) + (anti-TB) (V) |                        | 0.10 ± 0.03**                     | 0.646 ± 0.09*             | 3.60 ± 0.31*                            | 5.85 ± 0.72**           |

Notes: Each value is represented as mean ± SEM, No. of animals (n) = 6, *p < 0.01 when toxic control compared with control, ns = non-significance, *p < 0.05, **p < 0.01 versus toxic control, One way ANOVA followed by Dunett’s Test.
this plant extract might be beneficial to the individuals on anti-TB drugs. It could be proposed that the free radical scavenging activity of this herbal drug could be one of the possible mechanisms of action of hepatoprotection against drug induced hepatotoxicity. However, further studies are recommended to study the exact mechanism of hepatoprotective activity of this commonly used ingredient of culinary preparations having high potential of therapeutic implications.

Supplementary material
Supplementary material relating to this paper is available online.

Disclosure statement
No potential conflict of interest was reported by the authors.

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