SUPPLEMENTARY MATERIAL

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

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Materials and methods

Plant collection and identification

Fresh fruits of *T. indica* were purchased from the local market of New Delhi, India. The plant material was identified and authenticated by the taxonomist of Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi, India. A voucher specimen (TI/FP-368) was deposited in the department of Pharmacognosy and Phytochemistry for future reference.

Chemicals

All chemicals used in the study were of analytical grade and purchased from S.D. Fine Chemicals Ltd., India. 1,1-diphenyl-2-picrylhydrazyl (DPPH), Silybon 70[®] (standardized extract of silymarin), Isoniazid and Rifampicin were purchased from Sigma–Aldrich (USA), Microlabs; Holar, TN, India and Panacea Biotech Limited (New Delhi, India), respectively.

Preparation of extracts of T. indica

The fruits of *T. indica* were washed with distilled water to remove dirt and other impurities. It was dried in hot air oven at 40 -50°C and then powdered using kitchen mixer grinder to obtain a coarse powder. The fruit powder of *T. indica* (100 gm) was extracted using double distilled water (1000 mL) for 6 h. The soaked powdered material was kept overnight with occasional shaking and sonicated for 30 min at 25 ± 3°C in ultra sonicator water bath. It was filtered and dried in rotary evaporator at 45°C. The aqueous fruit extract of *T. indica* (AFTI) was stored at 4°C temperature and used as such for further studies.

Determination of in vitro antioxidant activity by RP-HPLC-DPPH method

The free radical-scavenging activity of the AFTI was investigated by using reversed phase-HPLC-DPPH method as reported earlier (Yen and Chen 1995). The DPPH solution (1 mL, 100 µM) was mixed with 1 mL of extract or positive control (Ascorbic acid) and 3 mL of double distilled water was added to this mixture. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 10 min. The decrease in peak area of the resulting mixture was monitored at 517 nm for 10 min. The difference in the reduction of peak area between the control (DPPH in double distilled water) and the sample was used for calculating the % free radical inhibition of the sample. The experiment was performed in triplicate.

The sample for HPLC was prepared by filtering through 0.2µm Nylon membrane filter and an aliquot (20 µL) of the sample is injected for reversed phase- HPLC analysis. The HPLC system

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consisted of a Shimadzu HPLC system (LC-10 Ai, Japan) a pump (LC-10 Ai), a system controller (SCL-10AVP), and a diode array detector (SPD-M10 AVP). Data analysis and processing were done by class LC10 software (Version 1.6). Analyses were carried out on a LiChrospher® 100 RP-18e column (250mm×4 mm, 5µM) (Merck, Darmstadt, Germany). Isocratic elution with methanol/water (80:20 v/v) was carried out at a constant flow rate of 1 mL/min.

$$\text{Radical Scavenging (\%)} = \frac{(\text{PA}_{\text{Control}} - \text{PA}_{\text{Sample}})}{\text{PA}_{\text{Control}}} \times 100$$

Where,

PA_{Control} = Peak area of control

PA_{Sample} = Peak area of sample

Animals

The animal study was approved by the Institutional animal ethical committee of Jamia Hamdard (registration no 173/CPCSEA) and all the experiments were performed as per the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA). Healthy Wistar albino rats of either sex, having weight in the range of 150–200 gm were used for the evaluation of hepatoprotective activity. The animals were sheltered under hygienic conditions in polypropylene cages at the animal house facility of Faculty of Pharmacy. One week before the commencement of study and during all the experiments, all animals were kept at temperature maintained at $24 \pm 1^{\circ}\text{C}$; relative humidity 45-55% with alternate 12 h of light and dark cycle. The animals were allowed easy access to standard rodent diet and water *ad libitum*.

Acute toxicity

Acute toxicity study of AFTI was conducted in adult Wistar Albino rats as per the OECD guideline number 423 (OECD, 2002). Animals were divided into test groups having six animals in each group. An increasing dose of herbal extract from 100-3000 mg/kg body weight was administered *p.o.* to test animals (Ghosh, 1984). The rats after treatment were continuously observed for 1 h and then after every half an hour up to 4 h and any change in gross behavior, general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, fecal output, feeding behavior etc, were noted down. The rats were further observed up to 72 h for any mortality. The extract was found to be non toxic and safe in rats up to 3000 mg/kg by

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oral route, as no significant behavioral changes and mortality was observed in rodents at any tested dose level.

In vivo study protocol

Albino rats were randomly divided into five groups comprising six animals in each group. Hepatotoxicity was induced by using combination of two anti-tubercular drugs, INH and RIF. All groups except normal control group were administered INH (50 mg/kg) and RIF (100 mg/kg) intraperitoneally (*i.p.*) on the first day of study to induce liver toxicity (Sodhi et al., 1998; Tasaduq et al., 2005).

The Group I served as the normal control group and received normal saline only for 14 days. Group II was toxic control representing hepatotoxicity model group that received normal saline (1 mL/kg, *p.o.*) for 14 days.

The third group (Group III) was positive control group and was treated with silymarin (25 mg/kg, *p.o.*) for 14 days.

Groups IV and V were treated with aqueous extract of *T. indica* (250 mg/kg and 500mg/kg, *p.o.*) for 14 days, respectively.

Blood sample collection

On the 15th day, the animals were anesthetized by exposing to vapours of ether and their blood samples were collected in the sterile centrifuge tubes by cardiac puncture immediately and allowed to clot. The livers of animals were also preserved for biochemical estimation and histopathological examinations.

Analysis of biochemical parameters

Blood samples obtained by cardiac puncture were centrifuged to separate the serum for biochemical analysis. Serum was analyzed to determine level of liver biochemical markers such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), albumin, total bilirubin, alkaline phosphatase (ALP) (Dumas, 1971; Kind & King, 1954; Malloy & Evelyn, 1937; Reitman & Frankel, 1957). Antioxidant parameters estimated in homogenate hepatic tissue by standard reported methods include; thiobarbituric acid reactive substances (TBARS), Glutathione (GSH) level, Superoxide Dismutase (SOD) and Catalase (CAT) (Claiborne 1985; Marklund & Marklund, 1974; Ohkawa et al., 1979; Sedlak & Lindsay, 1968).

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Histopathological investigation

10% formaldehyde was used to preserve the small pieces of rat liver tissues and later on these were embedded into paraffin blocks to obtain the thin sections for histopathological investigations. Thin sections (5 μ M) of liver tissues were routinely stained with haematoxylin and eosin (H&E) dyes and examined under a light microscope (Luna, 1968).

Data analysis

All the experiments were performed in triplicate and the results are expressed as mean $\pm$ S.D. One-way analysis of variance (ANOVA) followed by Dunnet's post-hoc test was used to analyze the total variation present in a set of data. $P < 0.05$ and $P < 0.01$ were considered to be statistical significant.

Figure Legends:

Figure S1: (a) HPLC Chromatograms of DPPH as control; (b) HPLC Chromatograms of DPPH with Ascorbic Acid; (c) HPLC Chromatograms of DPPH with *T. indica* extract

Figure S2: (A) High power photomicrograph of liver from normal control group showing a portal triad with normal structures, PV = Portal Vein, BD = Bile Duct (HE x 400).

Figure S2: (B) High power photomicrograph of portal triad area of liver tissue from toxic group animal showing vacuolated hepatocytes and mild periportal inflammatory cell infiltration (HE x 400).

Figure S2: (C) High Photomicrograph from standard animal showing normal liver cells and sinusoids in the centrizonal area. CV= Central Vein (H&E 400x).

Figure S2: (D) High power photomicrograph of Liver from *T. indica* (250 mg/kg) group showing the portal triad with moderate degree of inflammatory cell infiltration around the bile duct. PV = Portal Vein, BD = Bile Duct (HE x 400x)

Figure S2: (E) High power photomicrograph of Liver from *T. indica* (500 mg/kg) group showing a portal triad with moderate degree of inflammatory cell infiltration around the bile duct

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Figure S1(a-c)

Figure S2 (A-E)

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