ORIGINAL ARTICLE

Hepatoprotective activity of aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* (Aiton) Hassk against antitubercular drugs induced hepatic damage

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Background: *Rhodomyrtus tomentosa* of family Myrtaceae has been used traditionally in Chinese medicine for the treatment of liver disorders. However, there is no scientific evidence on the hepatoprotective potential of the plant. **Aim:** The present study was aimed at evaluating the hepatoprotective and antioxidant potential of the aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* leaves (RTLE) based on its traditional claim in liver diseases. **Materials and Methods:** Hepatotoxicity was induced in albino rats by administering a combination of three antitubercular drugs, Isoniazid (7.5 mg/kg), Pyrazinamide (35 mg/kg) and Rifampicin (10 mg/kg) orally for 45 days. Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine aminotransferase (ALT), total protein and total bilirubin (TB) levels were estimated in serum samples using diagnostic kits. Antioxidant parameters were estimated in liver homogenates using standard methods. **Statistical Analysis:** The experimental mean values were compared statistically with that of toxicant group by using One-way ANOVA followed by Newman-Keul's multiple tests. **Results:** Hepatoprotective activity of the extract was evident from the significant decrease in the elevated levels of ALT, AST, ALP and total bilirubin and an increase in the levels of total protein in comparison to toxicant control. Increased activity of antioxidant enzymes supports the *in vivo* antioxidant activity of the extract. Histopathological changes of the liver in the treated and control group of rats were in agreement with the hepatoprotective finding. **Conclusion:** RTLE exhibited hepatoprotective activity against antitubercular drug induced hepatotoxicity through a free radical scavenging effect and reduces oxidative damage caused by antitubercular drugs.

Key words: Antioxidant enzymes, antitubercular drugs, hepatoprotective, *Rhodomyrtus tomentosa*

INTRODUCTION

Oxidative stress induced damage to hepatocytes has been found to have a key role in antitubercular drugs induced hepatitis.[1] Alterations of various cellular defence mechanisms consisting of enzymatic and non-enzymatic components have been reported in INH, PZA and RIF induced hepatotoxicity.[2]

Rhodomyrtus tomentosa (Ceylon hill gooseberry) of family Myrtaceae is traditionally used for wounds and abscesses.[3] *In vitro* antioxidant activity of the different extracts of *Rhodomyrtus tomentosa* has been reported by different methods.[4] Natural antioxidants are known to exert beneficial effects in hepatitis induced by antitubercular agents[5] by preventing the attack by free radicals or reactive oxygen species (ROS). Hence the present study was conducted with an aim to evaluate the hepatoprotective and antioxidant potential of the aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* leaves based on its traditional claim in liver diseases.

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MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Rhodomyrtus tomentosa* was collected in the month of April/June from Nilgiris, Ootacamund, Tamil Nadu. The plant was identified, confirmed and authenticated by Field Botanist, Dr. S. Rajan. The shade dried leaves was then ground into a coarse powder by a mechanical grinder and was used for extraction of phytoconstituents.

Preparation of Extracts

The powdered leaves were subjected to hot successive extraction in a soxhlet apparatus with petroleum ether, chloroform, ethyl acetate, aqueous alcohol (70%) and water.[6] The extracts were filtered and dried under

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vacuum in a rotary flash evaporator and stored in a refrigerator until use

Phytochemical Screening

Preliminary phytochemical screening of aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* leaves was performed to detect the presence of alkaloids, phenolics, flavonoids, saponins, carbohydrates, steroids and terpenoids.[7]

Estimation of Total Phenolic Content

Total phenolic content present in the extractives was measured by Folin-Ciocalteu assay (FC) using Gallic acid as the calibration standard to express results in mol^{-1} or Gallic acid equivalents (GAE).[8]

Animals

Albino rats of Wistar strain of either sex weighing between 150-200 g were used. They were housed in standard cages at room temperature (25±2°C) and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but had free access to drinking water. The study was conducted after obtaining Institutional ethical committee clearance and bears the approval number, DSCP/M.Pharm.col/IAEC/32/10-11.

Drugs and Chemicals

Chemicals of standard grade were purchased from S.D Fine Chemical Pvt. Ltd. India, Merck Specialties Pvt. Ltd. India. Rifampicin, Isoniazid and Pyrazinamide were obtained as gift samples from Microlabs, Bangalore.

Acute Toxicity Studies

Six rats were fasted overnight and were administered a single oral dose (2000 mg/kg, *b.w.*) of the aqueous alcoholic extract of *Rhodomyrtus tomentosa*.[9] After the administration of the extract, food was withheld for further 3 to 4 hrs. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily, cage side observations including changes in skin and fur, eyes and mucous membrane etc., were observed. Mortality, if any, was determined over a period of 2 weeks.

Evaluation of Hepatoprotective Activity

Hepatoprotective activity of aqueous alcoholic extract of *Rhodomyrtus tomentosa* was evaluated against antitubercular drug induced hepatotoxicity in rats.[10] Isoniazid (7.5 mg/kg) and pyrazinamide (35 mg/kg) were dissolved in sterile distilled water whereas Rifampicin (10 mg/kg) was first dissolved in 0.5 ml of 0.1N HCl and then made up to the required volume with sterile distilled water. Aqueous alcoholic extract of *Rhodomyrtus tomentosa* was suspended in 0.3% CMC. Silymarin suspension was prepared by suspending the pure sample of Silymarin in sterile distilled water. All drugs including the extracts under study and the standard drug were given orally once daily for 45 days by gastric incubation.

Treatment Protocol of Various Groups (G) of Animals

G1 - Normal control (0.3% CMC, 10 ml/kg *b.w.*); *p.o.*
G2 - Toxicant control (INH 7.5 mg/kg, RIF 10 mg/kg and PZA 35 mg/kg); *p.o.*
G3 - Standard - Silymarin (200 mg/kg) + (INH + RIF + PZA); *p.o.*
G4 - RT extract (100 mg/kg) + (INH + RIF + PZA); *p.o.*
G5 - RT extract (200 mg/kg) + (INH + RIF + PZA); *p.o.*
G6 - RT extract (400 mg/kg) + (INH + RIF + PZA); *p.o.*

The animals were treated as per the above protocol for 45 days.

Biochemical Estimation

On the 45th day blood was collected by retro orbital puncture 1 h after administration of drugs and centrifuged at 3000 rpm. Alkaline phosphatase (ALP), Aspartate amino transferase (AST), Alanine aminotransferase (ALT), total protein and total bilirubin (TB)[11] levels were estimated in serum samples with diagnostic kits obtained from Coral Biosystems, Goa, India using a semiautoanalyzer.

Antioxidant Parameters

Animals were sacrificed after collection of blood samples and the liver was dissected out, washed in ice cold saline and a homogenate was prepared in 0.3 M phosphate buffer, pH 8.4. The homogenates were centrifuged and supernatant was used for the assay of marker enzymes, superoxide dismutase (SOD)[12] and Catalase (CAT).[13] Lipid peroxide levels (LPO)[14] were estimated in terms of thiobarbituric acid reacting substances using standard methods.

Histopathological Studies

The liver was excised quickly and fixed in 10% formalin at room temperature. After dehydration using graded ethanol, pieces of tissues were embedded in paraffin, cut in fine (5 µm) sections and mounted on glass slides. Sections were then deparaffinized with xylene, counterstained with hematoxylin and eosin and viewed under a light microscope at ×400 for degeneration, fatty changes and necrotic changes.

Statistics

The results were expressed as Mean ± SEM. The experimental mean values were compared statistically with that of toxicant group by using One-way ANOVA followed by Newman-Keul's multiple tests. The analysis was carried out using Graph Pad Prism software V.4. *P* and values less than 0.5 were considered to be statistically significant.

RESULTS

In the qualitative phytochemical analysis, carbohydrates, terpenoids, steroids, tannins, saponins and flavonoids were found to be present in aqueous alcoholic extract of RT leaves. Phenolic content present in each extract of *Rhodomyrtus tomentosa* leaves was expressed in terms of Gallic acid and was found to be 0.44±0.68, 1.24±0.22, 3.24±0.20, and 4.02±0.24 mg GAE/g dry weight of the petroleum ether, chloroform, ethyl acetate and aqueous alcoholic extractives respectively [Table 1].

The extract at dose levels of 500, 1000 and 2000 mg/kg *b.w.* did not show any significant behavioural alterations and toxicity in the initial four hours. 72 h observations showed no mortality up to a dose level of 2000 mg/kg *b.w.* Antitubercular drug induced hepatotoxicity in rats resulted in a marked increase in TB, serum AST, ALT, ALP and a decrease in total protein level. Pre-treatment with RT extract at 100 and 200 mg/kg showed protective effect as it caused significant decrease in the levels of TB, AST, ALT, ALP and increased total protein level. Protective effect of RT extract at 400 mg/kg was comparable with the standard drug Silymarin at 200 mg/kg [Table 2].

Histological profile of the normal control animals showed normal architecture with central veins and radiating cords of hepatocytes. Toxicant control animals which received antitubercular drugs alone exhibited degenerating hepatocytes. The parenchyma showed areas of necrosis with mixed inflammatory infiltration and periportal infiltration by mixed inflammatory cells comprising of lymphocytes and neutrophils. Animals treated with RT extract at 100 and 200 mg/kg exhibited regeneration of hepatocytes. The parenchyma showed few mononuclear inflammatory cells. Animals treated with RT extract at 400 mg/kg and standard drug, Silymarin at 200 mg/kg showed hepatic architecture, which was nearly similar to that of healthy control [Figure 1].

Table 1: Phenolic content present in the extractives of *Rhodomyrtus tomentosa* leaves

Sample	Phenolic content in terms of Gallic acid (mg/g*)
Petroleum ether extractives	0.44±0.68
Chloroform extractives	1.24±0.22
Ethyl acetate extractives	3.24±0.20
Aqueous alcoholic extractives (70%)	4.02±0.24

*All values are average of three determinations, mean±SEM

DISCUSSION

Increase in the level of lipid peroxides in liver reflects hepatocellular damage. The depletion of antioxidant defences and/or raise in free radical production deteriorates the pro-oxidant antioxidant balance, leading to oxidative stress induced cell death. Oxidative liver damage mediated by antitubercular drugs is generally attributed to free radical generation, which act as inducer of lipid peroxidation and source for destruction and damage to the cell membrane.[15] Indeed, RT extract supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that RT-extract possibly has antioxidant property to reduce antitubercular drugs induced membrane lipid peroxidation. Recent studies have shown that antioxidant natural compounds increases the activity of antioxidant enzymes.[16]

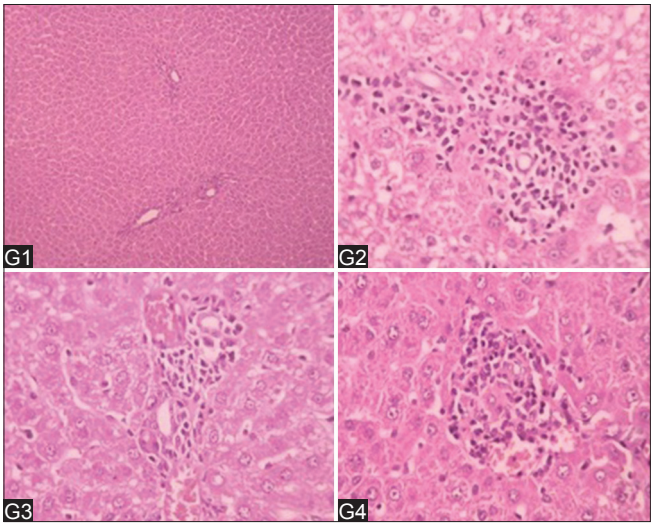

Figure 1: Representative photomicrographs (400×) of histopathological changes of liver showing the effect of *Rhodomyrtus tomentosa* extract on antitubercular drug induced hepatotoxicity (G1) Normal control. (G2) Treated with antitubercular drugs. (G3) Pretreated with Silymarin (200 mg/kg p.o). (G4) Pretreated with RT extract (100 mg/kg p.o.).

Table 2: Effect of RT extract on different biochemical parameters in INH+PZA+RIF induced hepatotoxic rats

Group	AST (U/dl)	ALT (IU/dl)	ALP (IU/dl)	Total bilirubin (mg/dl)	Total protein (gm/dl)
Normal control	16.04±0.56	9.49±0.53	370.3±7.3	0.22±0.004	6.80±0.008
Toxicant control	29.30±0.50	17.49±0.32	875±13.1	0.39±0.003	4.88±0.029
Standard	16.04±0.48***	9.62±0.41***	370.8±5.4***	0.24±0.007**	6.74±0.01**
RT extract (100 mg/kg)	22.10±0.42**	14.17±0.26**	607.2±6.8**	0.32±0.004**	5.52±0.008**
RT extract (200 mg/kg)	19.20±0.30**	12.57±0.30**	452.6±1.2**	0.31±0.01**	5.89±0.006**
RT extract (400 mg/kg)	15.93±0.48***	9.53±0.32***	366.4±8.1***	0.23±0.005**	6.79±0.02**

Values are expressed in terms of mean±S.E.M (n=6), **P<0.01, ***P<0.001 vs. toxicant control

Table 3: Effect of RT extract on antioxidant parameters in INH+PZA+RIF induced hepatotoxic rats

Groups	CAT (unit/min/mg protein)	SOD (unit/min/ mg protein)	LPO (nmole of MDA/mg protein
Normal control	128.64±5.64	9.77±0.21	0.68±0.081
Toxicant control	71.35±3.35	5.53±0.32	1.32±0.12
Standard	113.83±5.18***	9.29±0.17**	0.70±0.066*
RT extract (100 mg/kg)	81.63±4.39**	6.41±0.48*	1.13±0.125*
RT extract (200 mg/kg)	89.27±4.21**	7.86±0.45**	0.96±0.065*
RT extract (400 mg/kg)	107.48±5.9**	9.18±0.25**	0.75±0.061*

Values are expressed in terms of mean±S.E.M (n=6), *P<0.05, **P<0.01, ***P<0.001 vs. toxicant control ; CAT - Catalase; SOD - Superoxide dismutase; LPO - Lipid peroxide levels

Our study revealed that chronic exposure to antitubercular drugs decreased the activities of the ROS scavenging enzymes, viz. SOD and Catalase. This decreased activity of SOD and Catalase is due to formation of hepatotoxin metabolite hydrazine directly from INH or indirectly from acetylhydrazine.[17] Rifampicin induces cytochrome P450 enzyme causing an increased production of the toxic metabolites from acetyl hydrazine (AcHZ).[18] Pre-treatment with RT extract restored the activities of both these antioxidant enzymes to near normal values possibly by reducing generation of free radicals and hepatocellular damage [Table 3].

Antioxidant activity of natural phenolic compounds has been reported.[19] Hence, the antioxidant activity of the extract may be attributed to the phenolic compounds present in the extract.

In conclusion the aqueous alcoholic (70%) extract of *Rhodomyrtus tomentosa* exhibit hepatoprotective activity against antitubercular drug induced hepatotoxicity through a free radical scavenging effect and reduces oxidative damage caused by antitubercular drugs. A further detailed study on various other parameters is required to elucidate the exact mechanism of action.

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