Hepatoprotective activity of methanolic extract of *Mussaenda philippica* (stems) against anti-tubercular drugs induced hepatotoxicity

D. Swarnalatha*, G. Sowjanya, Chakka Gopinath, T. S. M Saleem

Annamacharya College of Pharmacy, Rajampet-516126, Kadapa, Andhra Pradesh, India.

**Abstract**

The present study was designed to evaluate the hepatoprotective effect of methanolic extract of *Mussaenda philippica* against isoniazid and rifampicin induced hepatotoxicity in experimental rats. Six groups of six rats were selected for the study. Methanolic extract of *Mussaenda philippica* at a dose of 100, 200 and 400 mg/kg as well as silymarin (50 mg/kg) were administered orally once daily for 14 d in isoniazid and rifampicin group. The serum levels of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase, and bilirubin were estimated along with total protein. Histopathological analysis was carried out to assess injury to the liver. The altered biochemical parameters were significantly reverting back by the methanolic extract treatment. Histopathology also supported the biochemical variation. From this study it has been concluded that the methanolic extract of *Mussaenda philippica* shows significant hepatoprotective activity.

**Keywords:** *Mussaenda philippica*, anti-tubercular drugs, hepatoprotective activity, Methanolic

1. Introduction

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions such as drug metabolism, amino acid metabolism, lipid metabolism and glycolysis. Liver is capable of detoxifying toxic substances and synthesizing useful ones.1 Hepatotoxic agents can cause very serious damages to the liver as they may deprive the liver from its principal functions.2 Hepatotoxic chemicals cause the liver damages which are induced by lipid peroxidation and other oxidative damages.3,4 Many traditional remedies employ herbal drugs for the treatment of liver ailments. In India, a number of medicinal plants and their formulations such as *Aegle marmelos*, *Solanum nigrum* and *Ficus carica* are used to cure hepatic disorders in traditional medicine. *Mussaenda philippica* is unexplored medicinal plant and traditional practitioners using the crude extract of this plant for liver disorders. To support the traditional claim the present study was designed to evaluate the hepatoprotective activity of methanolic extract of *Mussaendaphilippica* against anti-tubercular induced hepatotoxicity in experimental rats.

2. Materials and methods

2.1 Chemicals and drugs

Isoniazid and rifampicin were purchased from Sigma- Aldrich Company (USA). Silymarin was obtained from Ranbaxy Laboratories Limited, India. All other chemicals were of analytical grade. Serum SGOT, SGPT and ALP were determined by kinetic method using the kit of Span Diagnostic Ltd., India in a double beam spectrophotometer.

2.2 Plant material

The stems of *Mussaendaphilippica* was collected in 2012 from Thalakona and Tirumala, Chittoor district, Andhra Pradesh. Botanical identification of the plants was done by Dr. K. MadhavaChetty, Department of Botany, S.V. University, Tirupati. Voucher specimen (ANCP-Plant-12/2014) was submitted to parent department.

2.3 Animals

Wistar albino rats of either sex weighing 150-200 g were kept at departmental animal house of Annamacharya College of Pharmacy, Rajampet, at a temperature (25°C ± 2) and 12 h light/dark cycle respectively for one week before and during the experiments and fed with standard diet and water *ad libitum*. Animal studies were conducted according to the Institute AnimalEthics Committee (1220/a/08/CPCSEA) regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

2.4 Preparation of extract

The dried stem samples were cut into small pieces and reduced to coarse powder by mechanical grinder, extracted with methanol as solvent in soxhlet extractor for 72 h. The extract was filtered and concentrated under reduced pressure.
using rotavapor. The percentage yield of extract is 10%. The extract was freeze-dried and stored in deep freezer for further use.

2.5 Preliminary phytochemical screening

The methanolic extract obtained was tested for the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar by Trease and Evans.

2.6 Acute oral toxicity study

The acute oral toxicity study was performed according to the method described by Lorke. Methanolic extract up to a dose of 2000 mg/kg did not produce any signs of toxicity and mortality. Based on this the doses for methanolic extract for further experimental study were selected.

2.7 Isoniazid and rifampicin induced hepatotoxicity

Isoniazid and rifampicin (50 mg/kg body wt. each, p.o) suspension were prepared separately in carboxy methyl cellulose (CMC). Rats were treated with isoniazid (INH), co-administered with rifampicin (RIF) for 14 d orally to produce hepatotoxicity.

2.8 Experiment protocol

Male wistar albino rats were divided into six groups comprising six animals in each group. Group I (NC) : Normal control
Group II (HC) : Hepatotoxic control received INH+RIF
Group III : Animals were given INH+RIF+ silymarin(50 mg/kg)
Group IV: Animals were given INH+RIF+ MEMP (100 mg/kg)
Group V: Animals were given INH+RIF+ MEMP (200 mg/kg)
Group VI: Animals were given INH+RIF+ MEMP (400 mg/kg)
All the treatments were given orally in CMC (1%) in distilled water (10 mL/kg) by means of orogastric cannula for 14 d. At the end of the treatment, blood samples of all animals were collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the assay of serum marker enzymes.

2.9 Biochemical determinations

The biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and serum bilirubin (SB) were assayed according to standard methods using an assay kit.

2.10 Histopathology

Liver samples from all animals were processed for light microscopy. Tissue sections were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin (H&E). The degree of liver damage was examined blindly by a liver pathologist under a light microscope. The sections were examined for the pathological findings of hepatotoxicity such as centrilobular necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

2.11 Statistical analysis

The data are expressed as mean ± S.E.M. of six observations. The results were obtained statistically analyzed by One way analysis of variance by using graph pad prism software version 5. Value P <0.05 was taken as the criterion of significance.

3. Results

3.1 Phytochemical screening

The methanolic extract was tested and showed presence of alkaloids, carbohydrates, saponins, flavanoids, anthocyanins and tannins.

3.2 Effect of methanolic extract on biochemical parameters

The results of hepatoprotective effects of methanolic extract of Mussaenda philippica on INH + RIF intoxicated rats are shown in Table 1. Administration of INH+RIF at a dose of 50 mg/kg significantly (P<0.001) increased the level of SGOT, SGPT, ALP and Total bilirubin and significantly (P<0.001) decreased the level of total protein. Treatment of methanolic extract of Mussaenda philippica at a dose of 200 and 400 mg/kg significantly reverted the altered biochemical parameters. Lower dose of Mussaenda philippica failed to revert the level of SGOT and SGPT clearly indicate that the protective effect of Mussaenda philippica was dose dependent. Maximum therapeutic efficacy has been observed with higher dose (400 mg/kg) of Mussaenda philippica. And the effect similar to reference standard silymarin.

3.3 Effect of methanolic extract on histopathology of liver

Histopathological analysis of the INH + RIF treated animal (Group 2) showed severe centrilobular necrosis, fatty infiltration and lymphocytes infiltration, inflammation also observed in the centrilobular region with portal triaditis. Hepatocytes of the normal control group (Group 1) showed a normal lobular architecture of the liver. Methanolic extract of Mussaenda philippica treated group at a dose of 100, 200 and 400 mg/kg showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. Silymarin treated group at a dose of 50 mg/kg showed normal hepatocytes and their lobular architecture was normal. The results were present in Figure 1.
Table 1: Hepatoprotective activity of MEMP by Isoniazid and Rifampicin induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>SGPT U/L</th>
<th>SGOT U/L</th>
<th>ALP U/L</th>
<th>TP mg/dl</th>
<th>TB mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>222.2±4.012</td>
<td>208.3±2.848</td>
<td>223.8±2.120</td>
<td>9.367±0.166</td>
<td>0.390±0.021</td>
</tr>
<tr>
<td>Group II</td>
<td>339.5±7.58*</td>
<td>260.2±8.631*</td>
<td>354.7±4.587*</td>
<td>5.672±0.062*</td>
<td>0.858±0.082*</td>
</tr>
<tr>
<td>Group III</td>
<td>243.0±3.812***</td>
<td>213.0±1.549***</td>
<td>225.0±5.013***</td>
<td>9.00±0.165***</td>
<td>0.53!±0.031***</td>
</tr>
<tr>
<td>Group IV</td>
<td>333.5±4.463ns</td>
<td>253.2±4.175ns</td>
<td>334.0±5.132**</td>
<td>7.383±0.245***</td>
<td>0.650±0.042*</td>
</tr>
<tr>
<td>Group V</td>
<td>307.8±4.861***</td>
<td>237.0±3.406**</td>
<td>309.0±4.892</td>
<td>8.250±1.105***</td>
<td>0.515±0.004***</td>
</tr>
<tr>
<td>Group VI</td>
<td>292.3±3.393***</td>
<td>216.5±2.349***</td>
<td>233.5±3.085***</td>
<td>8.983±0.087***</td>
<td>0.453±0.019***</td>
</tr>
</tbody>
</table>

All values are shown as mean ±SEM. One Way ANOVA followed by Dunnet’s Multiple Comparison Test. and n=6. *p<0.05, **p<0.01 and ***p<0.001 when compared with toxicant group. ns-nonsignificant.

4. Discussion

The liver is a vital organ and injured by number of chemicals and drugs. In the present study, hepatotoxicity model in wistar rats was successfully produced by administering INH and RIF (50 mg/kg per day each) orally. Increase in the normal upper limits in the measured serum transaminases of INH+RIF group on day 14 of the experiment was a biochemical indication of liver injury. During the metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetyl hydrazine). Hydrazine play a pivotal role in INH-induced liver damage in rats, which is consistent with the report by Sarichet al.INH is metabolized in liver primarily by acetylation and hydrolysis, and these acetylated metabolites are thought to be hepatotoxins. The combination of INH and RIF was reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation and cytochrome P450 was thought to be involved the synergistic effect of RIF on INH. Administration of methanolic extract of Mussaenda philippica at a dose of 200 and 400 mg/kg improved liver function by decreasing the serum SGOT, SGPT and ALP levels in hepatotoxic rats significantly, which is almost comparable to group treated with silymarin, a potent hepatoprotective drug used as reference standard. Total bilirubin, a byproduct of the breakdown of red blood cells in the liver, bilirubin is a good indicator of liver function. High levels will cause icterus (jaundice) and are indicative of damage to the liver and bile duct. Treatment with methanolic extract of Mussaenda philippica reduced the serum ALP as well as the total bilirubin levels in INH + RIF induced hepatic injury, indicating its protective effect over liver and improvement in its functional efficiency. Hepatocellular disintegrate and the inflammation in the liver was observed in the centrlobular region by histopathological
examination in INH + RIF treated groups. The above observations strongly indicate the hepatoprotective activity of methanolic extract of *Mussaenda philippica* against INH+RIF intoxicated rats.

**Acknowledgement**

Authors are thankful to AICTE, New Delhi for financial support under RPS scheme and management, Annamacharya College of Pharmacy for providing necessary facilities.

**Reference**