Hepatoprotective effects of Adenanthera pavonina (Linn.) against anti-tubercular drugs-induced hepatotoxicity in rats


Department of Pharmacology, Faculty of Pharmacy, Integral University, Lucknow 226026, Uttar Pradesh, India

Abstract

Objective: The aim of the current study was to evaluate the hepatoprotective action of the leaves of Adenanthera pavonina against isoniazid (INH) and rifampicin (RIF)-induced liver damage in experimental animals.

Methods: Five groups of six rats each were selected for the study. A methanolic (50%) extract of A. pavonina at a dose of 100 and 200 mg/kg as well as silymarin 100 mg/kg were administered orally once daily for 28 days in INH + RIF treated groups. The serum levels of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, total protein, albumin and lactate dehydrogenase (LDH) were estimated along with activities of superoxide dismutase (SOD), catalase, glutathione, thiobarbituric acid reactive substances (TBARS). Histopathological analysis was carried out to assess injury to the liver tissue.

Result: The methanolic extract of A. pavonina was safe up to a dose of 2000 mg/kg. The significantly elevated serum enzymatic activities of SGOT, SGPT, ALP, bilirubin and LDH due to INH + RIF treatment were restored to near normal in a dose dependent manner after the treatment with methanolic extract of leaves of A. pavonina. Also the increased level of total protein and albumin towards normal by extract of A. pavonina leaves. In the anti-oxidant studies a significant increase in the levels of glutathione, catalase and superoxide dismutase was observed. In addition, methanolic extract also significantly prevented the elevation of hepatic malondialdehyde formation in the liver of INH + RIF intoxicated rats in a dose dependent manner. The biochemical observations were supplemented with histopathological examination of rat liver sections.

Conclusions: These findings suggested that the methanolic extract of leaves of A. pavonina exhibited hepatoprotective effects against INH + RIF induced hepatic damage in rats as compared to standard drug silymarin.

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1. Introduction

Adenanthera pavonina Linn. (Family: Fabaceae) commonly known as red wood and red-bread tree, is a deciduous tree, 18–24 m tall, erect and 60 cm in diameter. Many species of Adenanthera, including A. pavonina, have been used as traditional herbal medicine against a variety of diseases including diabetes, lipid disorders, diarrhoea, haemorrhage from the stomach, haematuria and as anti-inflammatory agent in gout. Traditionally, the ground seed of A. pavonina is widely used for the treatment of various human ailments such as treatment of boils, inflammation, blood disorders, arthritis, rheumatism, cholera, paralysis, epilepsy, convulsion, spasm and indigestion. Phytochemically, the seeds contain an anti-inflammatory active principle, O-acetyl ethanolamine. The leaves possess octacosanol, dulcitol, glucosides of β-sitosterol and stigmasterol. The bark furnishes stigmasterol glucoside, and pods contain glycosides, saponins and steroids. A new five-membered lactone ring with an exo-cyclic double bond compound, pavonin was isolated from the methanol soluble part of A. pavonina. The methanol seed extract has also been reported to demonstrate anti-inflammatory and analgesic activities. The crude extract of A. pavonina showed blood pressure lowering effect antifungal, anti-oxidant and cytotoxic, anti-diabetic and anti-hyperlipidemic activities.

Liver is the most important organ concern with the biochemical activities in the human body. It regulates many important metabolic functions and hepatic injury is associated with alteration of these metabolic functions. The disorder associated with the liver is numerous and varied as it is the frequent target of number of toxicants. Although viral infection is one of the main causes of...
hazardous substances, hepatotoxins, excessive therapy, environmental pollutants, and chronic alcohol ingestions can also cause severe liver injury.

Many traditional remedies employ herbal drugs for the treatment of liver ailments.14 The current study was undertaken to evaluate the hepatoprotective effects of methanolic extract of *A. pavonina* leaves against isoniazid and rifampicin induced liver damage in Sprague-Dawley rats. Isoniazid and rifampicin (INH + RIF), being the first line drugs used as antituberculous chemotherapy, are known to be associated with hepatotoxicity.15,16

2. Materials and methods

2.1. Preparation of plant extract

The fresh leaves of *A. pavonina* were collected from the field area of Pallavaram, Chennai, India, in the month of June 2010. The plant specimen was authenticated by National Institute of Herbal Science, Plant Anatomy Research Center, Chennai, Tamil Nadu (Voucher specimen no. PARC/2011/954 & 955). 500 g of the coarsely powdered material was packed in muslin cloth and subjected to a Soxhlet extractor for continuous hot extraction with methanol (50%) for 72 h at 30 °C. Thereafter methanolic extracts of *A. pavonina* were filtered through Whatman paper no. 42 and the resultant filtrates were concentrated under reduced pressure and finally vacuum dried. The yield of the methanolic extract was 11.2% w/w.

2.2. Materials and animals

All solvents, chemicals, solutions and reagents used in the study were of analytical grade procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India; Fischer Inorganics and Aromatics Pvt. Ltd., Lucknow. Isoniazid and rifampicin were obtained as a gift sample from Lupin Drug Laboratory Limited, India. Silymarin was obtained from Ranbaxy Laboratories Limited, India. All biochemical estimation kits were obtained from Robonik diagnostic, Lifechem diagnostik and Span diagnostics kit (India) Ltd. Major instruments used for the study were Autoanalyser (Merck Microlab 200, M/s Vital Scientific, The Netherlands) and Spectrophotometer (160A UV–Vis, Shimadzu, Japan).

Male Sprague-Dawley (SD) rats weighing 150–200 g and Swiss albino mice (25–30 g) were kept in the departmental animal house of Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh (India) at a temperature of 25 ± 2 °C and 12 h light/dark cycle, respectively, for one week before and during the experiments. Animals were allowed to access standard rodent pellet diet (Dayal animal feed, Lucknow, India) and drinking water. Food was withdrawn for overnight before the experiment though water was allowed *ad libitum* and allocated to different experimental groups. The study was performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethics committee, CPCSEA, India (Reg. No. 1213/ac/2008/CPCSEA/II).

2.3. Toxicity studies

Acute toxicity study was performed for the methanolic extract of leaves of *A. pavonina* according to the Organisation for Economic Co-operation and Development guidelines-No. 423 (2001) for acute toxic classic method.17,18 Swiss albino mice of either sex were used for each step in this study. The animals were fasted for overnight with only water available, after which the extracts were administered intragastrically at different doses of 50 and 300 mg/kg. Food and water were withheld for a further 1–2 h after drug administration. Mice were closely observed for the initial 4 h after administration, and then once daily for 14 days to observe mortality. If mortality occurred in two of the three animals at any dose, then this dose was assigned as a toxic dose. If mortality occurred in one animal, then this same dose was repeated to confirm the toxic dose. If mortality did not occur, the procedure was repeated for further higher doses, i.e. 2000 mg/kg. One-tenth and one-twentieth of the maximum tolerated dose of the extract tested (2000 mg/kg) for acute toxicity did not show mortality and were selected for evaluation of the effect of *A. pavonina* 100 and 200 mg/kg for hepatoprotective effects.

2.4. 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 28 days orally to produce hepatotoxicity.19

2.5. Preparation of doses

A known quantity of methanol extracts was suspended in 1% (w/v) carboxy methyl cellulose with distilled water to make the respective stock solutions. Water was used to dissolve silymarin (100 mg/kg) with carboxy methyl cellulose. From these stock solutions, the doses (100 mg/kg and 200 mg/kg) of the extracts were prepared. The doses were prepared fresh each day.

2.6. Experiment design

Male Sprague-Dawley rats (150–200 g) were divided into five groups comprising six animals in each group.

Group I (NC): Normal control (1% CMC)
Group II (HC): INH + RIF (50 + 50 mg/kg, p.o. 28 days)
Group III (MEAP1): INH + RIF (50 + 50 mg/kg) + *A. pavonina* (100 mg/kg, b. wt, p.o. 28 days)
Group IV (MEAP2): INH + RIF (50 + 50 mg/kg) + (200 mg/kg) *A. pavonina* (200 mg/kg, b. wt, p.o. 28 days)
Group V (HCSD): INH + RIF (50 + 50 mg/kg) + Silymarin (100 mg/kg, b. wt, p.o. 28 days)

2.7. Assessment of liver function

2.7.1. Biochemical estimations

Twenty-four hours after administration of the last dose of the treatment schedule with drugs and extracts, the animals were euthanized by an overdose of diethyl ether. Whole blood was withdrawn from the rats by sino-orbital puncture after an overnight fast. The blood was allowed to coagulate at room temperature for 30 min and then centrifuged at 2000 rpm for 15 min for separation of serum. The serum was used for estimating the biochemical parameters viz., glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin, total protein, albumin and lactate dehydrogenase (LDH), albumin, total protein (TP), total bilirubin. The supernatant of the liver homogenate was used for the estimation of enzyme anti-oxidants like catalase (CAT),20 tissue glutathione by Ellmann method, superoxide dismutase (SOD) by a colorimetric method21 and thiobarbituric acid reactive substances (TBARS). The contents of malondialdehyde (MDA) were determined by the method of Chaurasia.22
Table 1
Effect of methanolic extracts of *A. pavonina* and different biochemical parameters in INH + RIF induced hepatotoxic rats.

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>SBL (mg/dl)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.628 ± 0.008</td>
<td>51.48 ± 0.834</td>
<td>79.172 ± 1.407</td>
<td>151.59 ± 3.389</td>
<td>340.87 ± 2.652</td>
<td>6.72 ± 0.072</td>
<td>2.94 ± 0.049</td>
</tr>
<tr>
<td>Hepatotoxic (INH + RIF)</td>
<td>1.79 ± 0.127*</td>
<td>162.83 ± 2.476**</td>
<td>146.41 ± 1.991*</td>
<td>290.36 ± 3.190*</td>
<td>577.79 ± 2.638*</td>
<td>3.72 ± 0.170*</td>
<td>1.420 ± 0.121**</td>
</tr>
<tr>
<td>MEAP 100 mg</td>
<td>0.88 ± 0.013*</td>
<td>93.95 ± 1.267*</td>
<td>90.131 ± 2.407*</td>
<td>175.10 ± 2.926*</td>
<td>358.12 ± 2.718*</td>
<td>4.77 ± 0.134*</td>
<td>2.09 ± 0.027*</td>
</tr>
<tr>
<td>MEAP 200 mg</td>
<td>0.83 ± 0.007*</td>
<td>74.07 ± 3.801**</td>
<td>84.143 ± 1.991*</td>
<td>164.68 ± 1.863**</td>
<td>352.45 ± 1.441**</td>
<td>5.50 ± 0.155**</td>
<td>2.25 ± 0.112**</td>
</tr>
<tr>
<td>Silymarin 100 mg</td>
<td>0.76 ± 0.026*</td>
<td>66.54 ± 3.590**</td>
<td>81.177 ± 1.467**</td>
<td>204.48 ± 2.028**</td>
<td>348.12 ± 2.718**</td>
<td>5.66 ± 0.219**</td>
<td>2.27 ± 0.054**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. *n* = 6.

*P* < 0.001, Hepatotoxic vs normal control.

**P* < 0.001, Standard treatment vs hepatotoxic.

*P* < 0.01, Treatment vs hepatotoxic.

*P* < 0.05, Treatment vs hepatotoxic.

2.7.2. Histopathological studies

Slices of liver from each of the five animals in all groups were preserved in 10% buffered neutral formalin (pH 7.4). The tissues were mounted in the laboratory by embedding paraffin sections of 5–10 μm size. These sections were then stained with haematoxylin–eosin dye. The degree of liver damage was examined by a pathologist of R. S. Diagnostic Centre, Lucknow, for observation under a low power microscope for any pathological changes. Centrilobular necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc. was noted.

2.8. Statistical analysis

A result of biochemical estimation has been expressed as Mean ± Standard Error of Mean (S.E.M). The values were subjected to One Way Analysis of Variance (ANOVA) using SPSS-16 (Statistical Package for the Social Sciences) software. The variance in a set of data has been estimated by Dunnett’s *t*-test.

3. Results

3.1. Acute toxicity study

Mice administered with methanolic extract of *A. pavonina* up to 2000 mg/kg did not show any abnormal behaviour, during initial 4 h after drug administration. No mortality was observed during 14 days after treatment with methanolic extract of *A. pavonina* in either sex.

3.2. Effect of methanolic extract on biochemical parameters

The results of hepatoprotective effects of methanolic extract of *A. pavonina* on INH + RIF intoxicated rats are shown in Table 1. Administration of INH + RIF at a dose of 50 mg/kg b. wt, p.o. each significantly (*P* < 0.001) elevated SGPT, SGOT, ALP, LDH and, Serum Bilirubin (SBL) activities when compared to the normal control. Treatment of methanolic extract of *A. pavonina* at a dose of 100 mg/kg and 200 mg/kg b. wt 1 h prior to INH + RIF administration significantly protected the elevation of transaminases and ALP activities towards normal. Serum Bilirubin (SBL) and LDH were significantly (*P* < 0.01) reduced by administration of *A. pavonina* at a dose of 100 mg/kg and 200 mg/kg as compared to hepatotoxic controlled rats. The protection was better on dose 200 mg/kg and a significant increase (*P* < 0.01) was observed in the levels, TP and albumin in the serum, against the hepatotoxic control group (Table 1).

3.3. Effect of methanolic extract on anti-oxidant parameters

Activities of hepatic SOD, CAT, glutathione and TBARS are presented given in Table 2. SOD and glutathione activities were significantly (*P* < 0.01) enhanced after the treatment of *A. pavonina* + INH + RIF treated group. However, the hepatic CAT activity was improved significantly (*P* < 0.01) when compared to the hepatotoxic control. Further the activity of GSH was enhanced and normalized in the *A. pavonina* + INH + RIF treated. Hepatic MDA level was significantly (*P* < 0.05) elevated in INH + RIF control group than the normal group. It was significantly reduced by administration of *A. pavonina* at a dose of 100 mg/kg and 200 mg when compare to hepatotoxic rats.

3.4. Effect of methanolic extract on histopathology of liver

Histopathological examinations of liver tissues were performed by the method of Belur et al, 1990. For histological examinations on the 28th day, the liver was isolated and preserved in 10% neutral buffered formalin. Histopathological observation of tissues was carried out in a Pathology laboratory, at R.S. Diagnostic Centre, Lucknow, India. After fixation, the tissues were embedded in paraffin, clear in xylene and dehydrated in descending series of ethanol. At least four cross-sections were taken from each tissue of 5 μm thickness and stained with haematoxylin and eosin (H&E).

Table 2
Effect of methanolic extract of *A. pavonina* in different parameters in INH + RIF induced hepatotoxic rats.

<table>
<thead>
<tr>
<th>Drug treatments</th>
<th>GSH (µg/mg)</th>
<th>CAT (µmol of H₂O₂ consumed min/mg of tissue protein)</th>
<th>SOD (units/mg tissue protein)</th>
<th>MDA (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.14 ± 0.009</td>
<td>16.13 ± 0.654</td>
<td>4.68 ± 0.274</td>
<td>25.42 ± 0.915</td>
</tr>
<tr>
<td>Hepatotoxic (INH + RIF)</td>
<td>1.61 ± 0.007*</td>
<td>8.66 ± 0.377**</td>
<td>1.48 ± 0.064*</td>
<td>72.92 ± 0.713*</td>
</tr>
<tr>
<td>MEAP 100 mg</td>
<td>2.00 ± 0.006**</td>
<td>11.04 ± 0.395*</td>
<td>2.33 ± 0.097*</td>
<td>62.47 ± 1.282*</td>
</tr>
<tr>
<td>MEAP 200 mg</td>
<td>2.07 ± 0.006**</td>
<td>12.63 ± 0.616**</td>
<td>3.72 ± 0.105**</td>
<td>39.57 ± 0.614**</td>
</tr>
<tr>
<td>Silymarin 100 mg</td>
<td>3.22 ± 0.008*</td>
<td>14.32 ± 0.544*</td>
<td>4.24 ± 0.163*</td>
<td>31.86 ± 1.190*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M.; *n* = 6.

*P* < 0.001, Hepatotoxic vs normal control.

**P* < 0.001, Standard treatment vs hepatotoxic.

*P* < 0.01, Treatment vs hepatotoxic.

*P* < 0.05, Treatment vs hepatotoxic.
Following two changes xylene washes of 2 min each, tissue sections were mounted with DPX mount. The slides were evaluated for histopathological changes and photomicrographs were taken using microscope system. H&E staining was used to visualize and differentiate between tissue components in normal and in hepatotoxic control model. Histological evaluation of the liver tissues in the hepatotoxic model rats at the magnification of 40x showed marked changes at the periphery. The degeneration and necrosis of liver cells, presence of pycnotic nuclei, granular cytoplasm and increase in intercellular spaces (Fig. 1B). Similar focal changes are also seen in the central areas. Microscopic examination on normal liver section shows intact mucosal lining of flattened epithelial cells (Fig. 1A). Mucosal glands are seen compactly arranged, consisting of cells with vesicular nuclei with nucleoli and abundant eosinophilic cytoplasm. These glands are separated by thin strands of fibro connective tissue. Basement membrane is thick and intact. Glands near basement membrane have more basophilic cytoplasm and few bundles of fibrous tissue and occasional blood vessels are also seen (Fig. 1A). Hepatotoxic group treated with silymarin 100 mg/kg, body weight as reference drug (Fig. 1C) shows intact mucosal lining of flattened epithelial cells. Mucosal glands are seen compactly arranged without any abnormality or any degenerative changes of hepatocytes. In rats group treated with AP extract in two different doses (Fig. 1D and E), shows marked changes at the periphery, granular cytoplasm and decrease in intercellular spaces as compared to hepatotoxic control rats. Liver sections with minimal degenerative changes of hepatocytes with minimal swelling and necrosed area. The treatment with above extracts showed that there is a significant reduction in tissue damage along with minimal evidence of inflammation. Histological examination of liver tissues in rats supplemented with A. pavonina extract at the dose of 200 mg/kg body weight showing nearly normal tissue architecture, absence of inflammatory cells in the central areas showing significant.

4. Discussion

In our study, the combinatorial effect of anti-tubercular drugs (INH + RIF) was used to induce hepatotoxicity in the experimental animals as an already established model.24 The model has been reported in literature to produce various grades of liver damage, including centrilobular necrosis, liver cell proliferation and suppression of anti-oxidant system.25 Many researchers have suggested that part of hepatocellular injury induced by combination of anti-tubercular drugs has been mediated through cytochrome P450.26 Reduction in hepatic anti-oxidant function has also been suggested as one of the other mechanisms for hepatotoxicity caused by anti-tubercular drugs.27 The combination of these two anti-tubercular drugs-induced hepatotoxicity, manifested mainly as hepatocellular steatosis and centrilobular necrosis, possibly associated with cholestasis, and it has been suggested that toxic isoniazid metabolites bind covalently to cell macromolecules in both animal and human case studies.28 During the metabolism of INH, hydrazine was produced directly (from INH) or indirectly (from acetyl hydrazine). From earlier study it was evident that hydrazine plays a crucial role in INH-induced liver damage in rats. The combination of INH and RIF was reported as higher rate of inhibition of biliary secretion, an increase in liver cell lipid peroxidation and cytochrome P450 was thought to be involved the synergistic effects of RIF on INH.

The serum levels of a number of studied hepatic enzymes behave as diagnostic indicators for hepatic injury.27 Increased levels of SGPT, SGOT, LDH and ALP in serum of the INH + RIF induced animals certainly indicate liver damage. An increase in the levels of these marker enzymes in serum was due to the leakage of the enzymes from liver as a result of tissue damage. On concurrent treatment with methanolic extract of A. pavonina at dose of 100 and 200 mg/kg respectively, the serum marker enzyme levels were near to normal indicating protection against liver damage (Table 1 & Fig. 1D, E). This protective effect could be possibly due to the reduction in the tissue damage brought by the methanolic extract of A. pavonina. The results were compared with the standard silymarin. It is a general perception that, the serum bilirubin levels are elevated in hepatic injury. A marked elevation was observed in serum bilirubin levels of INH + RIF induced rats, whereas total protein (TP) and albumin levels in the serum were markedly decreased. A reduction in synthesizing proteins was seen following

![Fig. 1. (A–E): Effect of methanolic extracts of A. pavonina against anti-tubercular drugs (INH + RIF) induced histopathological changes in normal rat liver. (A) Normal rats showed normal hepatocytes with well preserved cytoplasm with normal lobular structural design of the liver, (B) anti-tubercular drugs (INH + RIF) induced rat liver, where white arrow indicates necrosis and black arrow indicates inflammation, (C) silymarin (100 mg/kg), (D) methanolic extract of A. pavonina 100 mg/kg, (E) methanolic extract of A. pavonina 200 mg/kg (H&E 40x).](image-url)
intoxication of the liver with hepatotoxicants. As seen in the silymarin treated group and methanolic extract of A. pavonina, all studied parameters were restored to normal condition from the abnormal ones.

Suppression of the anti-oxidant system in anti-tubercular drugs intoxicated rats has been reported earlier. The decreased activities of SOD and CAT, the primary anti-oxidant enzymes, are observed in the anti-tubercular drugs (INH + Rif) induced rats which may be due to the interaction of accumulated free radicals with the associated metal ions or with the active amino acids of these enzymes. In our study, the groups treated with methanolic extract of A. pavonina and silymarin, were found to restore the levels of anti-oxidant enzymes which could be due to the ability of the constituents in the administered compounds to scavenge reactive oxygen species. Hepatocellular disintegrate and the inflammation in the liver was observed in the centrifobular region by histopathological examination in INH + Rif treated groups. This could be only possible by the overall protective character of the extract. A plethora of reports has been published that flavonoids, alkaloids and saponins played a major role in protecting the liver from injuries. A. pavonina was found to be richly containing flavonoids and saponins. Thus the hepatoprotective effect of the methanolic extract of A. pavonina could be possibly due to the presence of flavonoids, alkaloids, glycosides and saponins.

5. Conclusion

The results of this study suggest that the methanolic extract of A. pavonina has protective effects against INH + Rif induced hepatic damage in experimental rats. Administration of the extract attenuates the hepatotoxic effects by decreasing MDA production and through an increase of anti-oxidant defences. Our study demonstrates the health benefits traditionally claimed to this medicinal plant in liver disease.

Conflicts of interest

All authors have none to declare.

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