**Hibiscus vitifolius** (Linn.) root extracts shows potent protective action against anti-tubercular drug induced hepatotoxicity

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**Abstract**

_Ethnopharmacological relevance:_ The roots of _Hibiscus vitifolius_ Linn. (Malvaceae) is used for the treatment of jaundice in the folklore system of medicine in India. This study is an attempt to evaluate the hepatoprotective activity of the roots of _Hibiscus vitifolius_ against anti-tubercular drug induced hepatotoxicity.

_Materials and methods:_ Hepatotoxicity was induced in albino rats of either sex by oral administration of a combination of three anti-tubercular drugs. Petroleum ether, chloroform, methanol and aqueous extracts of roots of _Hibiscus vitifolius_ (400 mg/kg/day) were evaluated for their possible hepatoprotective potential.

_Results:_ All the extracts were found to be safe up to a dose of 2000 mg/kg. Among the four extracts studied, oral administration of methanol extract of _Hibiscus vitifolius_ at 400 mg/kg showed significant difference in all the parameters when compared to control. There was a significant (*P* < 0.001) reduction in the levels of serum aspartate amino transaminase, alanine amino transferase, alkaline phosphatase, lactate dehydrogenase, total and direct bilirubin, where as an increase was found in the levels of total cholesterol, total protein and albumin. Liver homogenate studies showed a significant increase in the levels of total protein, phospholipids and glycogen, and a reduction in the levels of total lipids, triglycerides, and cholesterol against control animals. In the tissue anti-oxidant studies, we found a significant increase in the levels of catalase and superoxide dismutase, whereas there was marked reduction in the levels of thiobarbituric acid reactive substances, as compared to control. Histology of liver sections of the animals treated with the extracts showed significant reduction of necrosis and fatty formation when compared with control specimens.

_Conclusion:_ These findings suggest that the root extracts of _Hibiscus vitifolius_ have potent hepatoprotective activity, thereby justifying its ethnopharmacological claim.

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1. Introduction

*Hibiscus vitifolius* Linn. commonly known as tropical rose mallow, is an annual or biennial herbaceous bush found in the jungles and bushwoods distributed throughout the hotter parts of India and are commonly known as 'bharadwaji' and 'bankapas'. It belongs to the Malvaceae family. This plant is grown as a fiber crop in some parts of the world and the roots are reported to be useful for the treatment of head lice (Rao and Lakshminarayana, 1985), inflammation (Parmar and Ghosh, 1979) and pulsating anterior fontanelle (Arnold and Gulumian, 1984). The flowers are also reported for its anti-inflammatory, hypoglycemic (Thamizhiniyan and Subramanian, 2011), anti-bacterial (Maganha et al., 2010) activities and also for gynecological complaints (Steenkamp, 2003). Phytochemical investigations of the flowers of _Hibiscus vitifolius_ have revealed the presence of bioflavonoids such as gossypin and glucuronides of gossypetin such as hibifolin (Lai et al., 2009). Gossypin has been shown to suppress angiogenesis, inflammation and carcinogenesis (Kunnumakkara et al., 2007). It is also reported to have oral anti-oxidant property (Gautam and Flora, 2010), anti-hypercholesterolemic property (Lu et al., 2008) and anti-noiceptive property (Ramaswamy and Viswanathan, 1997). Moreover, Parmar and Ghosh (1979), also reported the effect of gossypin on the formation of galactose induced cataracts in rats.
As the vital organ in the body, liver plays a significant role for the metabolism of endogenous and exogenous agents. Even though there is a greater chance of liver damage occurs, drug elimination and detoxification has been done by the liver (Akindele et al., 2010). Hepatitis is one of the most common liver associated and prevalent diseases in the world. And in most hepatitis sufferers it seems to be associated with jaundice. Then again the drug related hepatotoxicity is a leading cause of hepatitis. In some instances the hepatitis progression has develops into irreversible cirrhosis and is associated with liver cancer (Wang et al., 2009). Regardless of the fact that hepatic problems are responsible for a significant number of deaths around the world, the current drugs regimen in clinics is not effective enough. Thus, it is crucial to develop new effective drugs to lessen the injury to the liver and to decelerate the development of liver injury to fibrosis, cancer and death.

The extract of *Hibiscus vitifolius* is traditionally used for the treatment of jaundice and associated liver damages in India (Vijayan et al., 2004; Kunnunakkara et al., 2007). However, no literature was found on the hepatoprotective action of the root extracts of *Hibiscus vitifolius*. Hibiscus species have hepatoprotective effects (Sunilson et al., 2008). Moreover, a large number of herbs and formulations in India have been claimed to have hepatoprotective activity. More than 93 well known plants are reported to be used in 40 patented and proprietary poly herbal formulations (Sharma et al., 1991; Agarwal, 2001). Despite tremendous advances, only a few significant and effective hepatoprotective agents e.g. silymarin, are available in modern therapeutics. Keeping this in view, the present study was aimed to evaluate the hepatoprotective activity of roots of *Hibiscus vitifolius* against hepatotoxicity induced by a combination of anti-tuberculal drugs based on the traditional claim.

2. Materials and methods

2.1. Plant material

The roots of *Hibiscus vitifolius* were collected from Kanyakumari district, Tamil Nadu, India. The plant material was authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Chennai, India. A voucher specimen of *Hibiscus vitifolius* (Herbarium number 10A5) was deposited in the library of Plant Anatomy Research Center, Chennai.

2.2. Preparation of root extracts of *Hibiscus vitifolius*

Fresh plant part was washed thoroughly with running tap water and then distilled water, before drying. Two kilo grams of the dried and coarsely powdered material was taken in an aspirator bottle and extracted successively by cold maceration with different solvents in the order of increasing polarity viz., petroleum ether, chloroform, methanol and water for six days. Five litters of each solvent were used to complete the extraction process. At the end of each extraction the mixture was filtered through Whatman® No. 41 filter paper (pore size 20–25 μm). All extracts were separately concentrated in a rotary vacuum evaporator, then weighed to calculate the yield and stored in a dessicator until further use. All the four extracts were examined individually by standard chemical tests to establish the nature of phytochemicals present in them.

2.3. Quantitative estimation of different parameters in the roots of *Hibiscus vitifolius*

Quantitative estimations viz., calcium content, ascorbic acid, β-sitosterol (Bumrela and Naik, 2011), phenols (Siddique et al., 2010), total free amino acids (Maity et al., 2009) and total carbohydrates (Kotkar et al., 2009) were also determined with the dry root powder of *Hibiscus vitifolius*.

2.4. Experimental animals

Healthy Wistar albino rats (150–200 g) and adult Swiss albino mice (20–25 g) of either sex, bred in the animal house of S.B. College of Pharmacy, Sivakasi, Tamilnadu, India were used in the study. The animals were maintained at standard conditions of temperature, humidity and light on standard pellet diet and water *ad libitum*. The study was carried out with the approval of the local Institutional Animal Ethics Committee [IAEC No: SBCP/F.7(f)/252 (b)].

2.5. Materials

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, Chennai; Loba Chemie Indo Austranel Co., Mumbai; Ranbaxy Laboratories Ltd., Punjab, India; Sigma Chemical Company, U.S.A. and Plethico Pharmaceuticals Ltd., Indore. All biochemical estimation kits were obtained from Ecoline, E-Merc (India) Ltd., MIDC, Taloga. Major instruments used for the study were Autoanlyser (Merck Microlab 200, M/s Vital Scientific, Netherland) and Spectrophotometer (160A UV-Vis, Shimadzu, Japan).

2.6. Acute toxicity studies of the extracts

Acute toxicity studies (Mukherjee et al., 2007) were carried on albino mice as per the guidelines (No. 423) given by the Organisation for Economic Co-operation and Development, Paris. Overnight fasted albino mice were divided into four groups of three animals each. Group I received petroleum ether extract; Group II received chloroform extract; Group III received methanol extract and Group IV received aqueous extract. The extracts were administered separately to all the three animals in each group at a starting single dose of 5 mg/kg. Animals were observed for a period of 2 h, then occasionally for 4 h for severity of any toxic signs and mortality (Miller and Tainter, 1944). When no mortality was observed the same dose would be additionally administered to one more animal for each group. If no mortality is observed at this dose, the same procedure would be repeated for dose levels of 50, 300 and 2000 mg/kg of extracts on separate newer groups. The LD₅₀ was thus determined and 1/5th of LD₅₀ value was taken as ED₅₀ value, which was selected for the hepatoprotective animal study. The animals were kept under observation up to 14 days after drug administration to find out any delayed mortality.

2.7. Preparation of doses

A known quantity of petroleum ether, chloroform and methanol extracts was suspended in 0.5% (w/v) carboxy methyl cellulose each separately to make the respective stock solutions. Plain distilled water was used to dissolve the water extract and the standard drug, silymarin (100 mg/kg). From these stock solutions, the doses (400 mg/kg) of the respective extracts were prepared. The doses were prepared fresh each day.

2.8. Protocol for hepatoprotective study

A total of 42 healthy rats, divided into seven groups of six rats each, were used in the study. A combination of Isoniazid (7.5 mg/kg), Rifampicin (10 mg/kg) and Pyrazinamide (35 mg/kg) (Ranbaxy Laboratories Ltd., India) mixed together in distilled water was used as the hepatotoxicant mixture (HPTM) to induce hepatotoxicity. Group I, which served as normal control received
vehicle. Group II served as a positive control which received no treatment but HPTM alone. Group III received 400 mg/kg of petroleum ether extract of *Hibiscus vitifolius* + HPTM; Group IV received 400 mg/kg of chloroform extract of *Hibiscus vitifolius* + HPTM; Group V received 400 mg/kg of methanol extract of *Hibiscus vitifolius* + HPTM; Group VI received 400 mg/kg of aqueous extract of *Hibiscus vitifolius* + HPTM and Group VII served as standard which received 100 mg/kg of silymarin + HPTM. All doses along with HPTM were administered for a period of 45 days.

2.9. Assessment of liver function

2.9.1. 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 thiopentone sodium. Whole blood was withdrawn from the rats by sino-orbital puncture after an over-night 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 further used for estimating the biochemical parameters viz., aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total cholesterol (TC), albumin, total protein (TP), total bilirubin and direct bilirubin.

The liver of all the animals were excised, washed with cold saline and blotted dry between filter papers. The livers were weighed and a known quantity was homogenized in 5 ml of 0.01M Tris–HCl buffer using glass Teflon tissue homogenizer. The homogenate was then used to determine total proteins with Folin Phenol reagent (Joseph and Raj, 2010), total lipids by the sulfo-phospho-vanillin reaction (James and Sampath, 2011), triglycerides (Abhilash et al., 2011), cholesterol (Firat and Kargın, 2010), phospholipids (Kuriakose and Kurup, 2011) and glycogen (Baranowski et al., 2011) levels. The homogenate was further centrifuged for 10 min at 2000 rpm to obtain the supernatant.

The supernatant of the liver homogenate was subjected for the estimation of enzymic anti-oxidants like catalase (CAT) (Tewari et al., 2011), superoxide dismutase (SOD) by a colorimetric method (Jaishree and Badami, 2010) and thiobarbituric acid reactive substances (TBARS) using determination of total antioxidant activity using this method was carried out using egg yolk homogenate (10%) in phosphate buffer (pH 7.4) as lipid rich media. (Hernández-Hernández et al., 2009).

2.9.2. Histopathological studies

Slices of the liver from each of the six animals in all groups were preserved in 10% buffered neutral formalin (pH 7.4). The tissues were mounted by Peter Fi’s double embedding paraffin sections of 5–10 μ size (Redman et al., 2010). These sections were then stained with haematoxylin–eosin dye and observed under a low power microscope for any pathological changes.

2.10. Statistical analysis

Results of biochemical estimation were 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. Minimum level of significance was fixed at 0.05.

3. Results

3.1. Extraction of the plant

Cold maceration of the powdered root material with petroleum ether, chloroform, methanol and water yielded 0.79%, 1.38%, 1.73% and 38.02% of respective extracts in semisolid consistency. Water extract was mucilaginous in nature. All extracts were yellow to yellowish brown in color. A preliminary phytochemical analysis of the extracts revealed the presence of proteins, steroids, glycosides, fats, tannins, triterpenoids, mucilage and flavonoids. Quantitative estimation of the whole root powder showed the presence of 0.52% ascorbic acid, 8.32% β-sitosterol, 0.73% phenols, 0.18% calcium, 58.62% carbohydrates and 8.14% total free amino acids.

3.2. Acute toxicity and assessment of liver function study

In acute toxicity studies, all the extracts were found to be safe up to 2000 mg/kg. No mortality or toxic symptoms were observed during the entire duration of the study. Among the four extracts studied for hepatoprotective activity, methanol and aqueous extracts of *Hibiscus vitifolius* showed maximum activity, which was comparable with the standard drug silymarin. However, methanol extract was found to be more potent than the aqueous extract, where a significant decrease (*P* < 0.001) was observed in the serum levels of AST, ALT, ALP, LDH, total bilirubin and direct bilirubin and a significant increase (*P* < 0.001) was observed in the levels of TC, TP and albumin in the serum, against the control group (Table 1).

Petroleum ether extract and chloroform extract of *Hibiscus vitifolius* were devoid of any hepatoprotective activity. A significant increase (*P* < 0.001) was observed against control, in liver homogenate parameters such as total protein, phospholipid and glycogen and a significant decrease (*P* < 0.001) in total lipids, triglycerides and cholesterol levels, on treatment with silymarin and methanolic extract of *Hibiscus vitifolius* (Tables 2 and 3).

3.3. Histopathological study of the liver

Histological profile of the normal animals showed normal hepatocytes with well-preserved cytoplasm, prominent nucleus, nucleolus and central vein. There was no sign of inflammation, fatty change and necrosis in these animals (Fig. 1A). In animals administered with HPTM alone, liver sections showed marked congested central veins, sinusoid and multifocal area of necrosis, fatty changes and inflammatory cell with granular swelling (Fig. 1B). Treatment with the methanolic extract of *Hibiscus vitifolius* showed greater reduction of necrosed area, with disappearance of inflammatory infiltrate around portal triad (Fig. 1C). Aqueous extract of *Hibiscus vitifolius* also showed reduction of the necrosed area and inflammatory cell infiltration around the central vein (Fig. 1D). No marked reduction was observed in petroleum ether extract and chloroform extracts at the same doses (Fig. 1E and F). However, silymarin showed no sign of necrosis (Fig. 1G).

A significant reversal (*P* < 0.001) against control was observed in the anti-oxidant levels of CAT, SOD and TBARS when treated with methanol extract and silymarin at 100 mg/kg.

4. Discussion

A wide variety of chemical compounds have been identified as hepatotoxins. The combination of anti-tubercular drugs (hepatotoxicant mixture or HPTM), which has been used in the present study, as a tool to induce hepatotoxicity in experimental animals, is a well-established model (Saraswathy et al., 1998). It produces various grades of liver damage, including centrilobular necrosis, liver cell proliferation (Graham et al., 2004) and suppression of
anti-oxidant system. Several researchers have suggested that part of hepatic injury induced by such combination of anti-tubercular drugs is mediated through cytochrome P-550 (Saraswathy and Devi, 2001). Reduced hepatic anti-oxidant function has also been suggested as one of the other mechanisms for hepatotoxicity caused by anti-tubercular drugs (Kale et al., 2003). Both animal and human case studies show that this combination induced hepatotoxicity manifests mainly as hepatic steatosis and centrilobular necrosis, possibly associated with cholestasis, and it has been suggested that toxic isoniazid metabolites bind covalently to cell macromolecules (Tostmann et al., 2008).

The hepatotoxicity induced with HPTM increased the levels of total lipids, triglycerides and cholesterol in liver homogenates of experimental animals in our study, which was in accordance with the observations of Seakins and Robinson (1963). Moreover, hepatotoxins like anti-tubercular drugs can also interfere with the hepatic phospholipids and protein synthesis (Santhosh et al., 2006) resulting a decrease in phospholipids and total protein levels as observed in our study. However, the phospholipid content in liver homogenate along with the other parameters viz. total protein, total lipids, triglycerides and cholesterol were retrieved to normalcy in methanolic extract treated group. This observation indicates the hepatoprotective potential of Hibiscus vitifolius. Marked depletion in hepatic glycogen levels in livers of experimental rats, caused by anti-tubercular drug mixture induced liver damage was observed previously (Pari and Kumar, 2002) and in our studies. Anti-tubercular drugs bring about a rise in free calcium, which may lead to glycogen mobilization. This was restored considerably after the treatment with methanolic extract of Hibiscus vitifolius, as it is thought to stimulate the liver cells to convert more glucose to glycogen, thus unearthing its hepatoprotective effect. Similar effects were observed in silymarin treated group. In all the parameters observed, in the whole study, aqueous extract was found best active after methanol extract.

The serum levels of a number of hepatic enzymes behave as diagnostic indicators for hepatic injury (Lee et al., 2010). Increased levels of LDH, AST, ALT and ALP in serum of the HPTM intoxicated rats and our study, whereas, the total cholesterol (TC), total protein (TP) and albumin levels in the serum were markedly decreased. In jaundice and parenchymatous liver diseases, serum levels of cholesterol will fall (Becker et al., 2008). A depression in synthesizing proteins is seen following intoxication of the liver with hepatotoxins. A similar observation was seen in our studies, where the levels of total protein and albumin were significantly decreased in the serum of the control animals. As seen in the silymarin treated group, following treatment with the methanolic extract of Hibiscus vitifolius, all the above mentioned parameters were restored to normalcy indicating the reversing the abnormal state to normal.

Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies (Newairy et al., 2009). Suppression of the anti-oxidant system in anti-tubercular drugs intoxicated rats has been reported earlier (Skakun and Silvka, 1992). The decreased activities of SOD

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of various extracts of Hibiscus vitifolius and silymarin on the biochemical parameters in serum of HPTM intoxicated rats.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>72.5 ± 0.57</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>46.8 ± 2.99</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>462.85 ± 2.99</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.16 ± 0.018</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.02 ± 0.001</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>3.21 ± 0.11</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>32.0 ± 0.26</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>76.8 ± 0.113</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>4.45 ± 0.45</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>368.61 ± 3.61</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>78.6 ± 0.37</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>40.1 ± 0.11</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>2.99 ± 0.09</td>
</tr>
<tr>
<td>Values are mean ± S.E.M. N=6, *P&lt;0.001, †P&lt;0.001, ‡P&lt;0.001, §P&lt;0.001 extracted treated groups vs. control groups.</td>
<td></td>
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</tbody>
</table>
and CAT, the primary anti-oxidant enzymes, are observed in the HPTM induced rats which may be due to the interaction of accumulated free radicals with the associated metal ions or with the active aminoacids of these enzymes (Yogeeta et al., 2007). During hepatotoxicity these enzymes are structurally and functionally impaired by the radicals resulting in liver damage. In our study, the groups treated with methanolic extract of Hibiscus vitifolius 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. Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in the levels of lipid peroxides in the supernatant of the liver homogenate of rats administered with HPTM is in accordance with the observations of Skakun and Slivka (1992). The increase in the TBARS of liver indicates enhanced lipid peroxidation leading to tissue injury and failure of the anti-oxidant defense mechanisms to prevent the formation of excess free radicals. The rats in our study which received methanolic extract of Hibiscus vitifolius retained the level of hepatic TBARS to near normal when compared to normal control. This shows the protective action of methanolic extract of Hibiscus vitifolius. The petroleum ether and chloroform extract did not show any significant activity.

A high degree of vacuolation followed by marked congested veins, degenerated nuclei, sinusoids, multifocal area of necrosis,
Table 2
The effects of various extracts of Hibiscus vitifolius and silymarin on the biochemical parameters in liver homogenate.

<table>
<thead>
<tr>
<th>Treatment groups (mg/kg)</th>
<th>Total protein mg/100 g</th>
<th>Total lipids mg/100 g</th>
<th>Triglycerides mg/100 g</th>
<th>Cholesterol mg/100 g</th>
<th>Phospholipids mg/100 g</th>
<th>Glycogen mg/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7462 ± 427</td>
<td>6268 ± 628</td>
<td>618.8 ± 91.5</td>
<td>609.2 ± 28.9</td>
<td>2415 ± 108</td>
<td>4945 ± 156</td>
</tr>
<tr>
<td>HPTM</td>
<td>4861± 313</td>
<td>8630± 185</td>
<td>922.8± 61.3</td>
<td>888.6± 39.4</td>
<td>1392± 68</td>
<td>965± 70</td>
</tr>
<tr>
<td>Petroleum ether 400 + HPTM</td>
<td>4980 ± 421</td>
<td>8311 ± 231</td>
<td>921.3 ± 72.3</td>
<td>896.5 ± 41.3</td>
<td>1463 ± 71</td>
<td>1632 ± 73</td>
</tr>
<tr>
<td>Chloroform 400 + HPTM</td>
<td>7402 ± 322</td>
<td>6018 ± 196</td>
<td>728.5 ± 36.4</td>
<td>679.3 ± 22.6</td>
<td>2360± 98</td>
<td>4571± 38</td>
</tr>
<tr>
<td>Methanolic 400 + HPTM</td>
<td>6806± 395</td>
<td>7213± 121</td>
<td>748.9 ± 46.7</td>
<td>703.6± 18.9</td>
<td>2265± 108</td>
<td>3564± 83</td>
</tr>
<tr>
<td>Silymarin 100 + HPTM</td>
<td>7614± 411</td>
<td>6769± 98</td>
<td>712.4 ± 46.3</td>
<td>668.8± 35.6</td>
<td>2386± 102</td>
<td>4747± 88</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M; N=6.

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