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Hepatoprotective effect of extracts from *Pergularia daemia* Forsk.

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Abstract

Pergularia daemia (Asclepiadaceae) is a perennial herb growing widely along the road sides of India. It has been used in folk medicine for the treatment of liver disorders. The aim of this work is to study the hepatoprotective effect of crude ethanolic and aqueous extracts from the aerial parts of *Pergularia daemia*. The aqueous and ethanolic extracts obtained from aerial parts of *Pergularia daemia* were evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride. The ethanolic extract at an oral dose of 200 mg/kg exhibited a significant ($P < 0.05$) protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and total cholesterol and increasing the levels of total protein and albumin levels as compared to silymarin used as a positive control. These biochemical observations were supplemented by histopathological examination of liver sections. The activity may be a result of the presence of flavonoid compounds. Furthermore, the acute toxicity of the extracts showed no signs of toxicity up to a dose level of 2000 mg/kg. Thus it could be concluded that ethanolic extract of *Pergularia daemia* possesses significant hepatoprotective properties.
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Keywords: *Pergularia daemia*; Asclepiadaceae; Carbon tetrachloride; Ethanolic extract; Silymarin

1. Introduction

Pergularia daemia Forsk. Syn. *Daemia extensa* (Asclepiadaceae) commonly known with the name of "dustapu teega" in Telugu is a perennial twining herb, growing wildly along the road sides of Andhra Pradesh state in India. The plant is used to treat jaundice by the folklore people of Chittoor district, Andhra Pradesh state. The literature survey reveals that little work has been carried out on this plant. The plant is useful as anthelmintic, laxative, anti-pyretic and expectorant, and is also used in infantile diarrhoea. This drug was also strongly recommended for malarial intermittent fevers (Kirtikar and Basu, 1983). Phytochemically the plant has been investigated for cardenolides, alkaloids, triterpenes and saponins (Sathish et al., 1998). Sathish et al. (1998) reported the anti-inflammatory, anti-pyretic and analgesic activities of the plant. The plant was also found to possess anti-diabetic activity (Wahi et al., 2002). The plant was found to contain various triterpenes and steroidal compounds (Anjaneyulu et al., 1998). The present study was undertaken to scientifically prove the folklore use of the plant against liver disorders.

2. Materials and methods

2.1. Plant material

The aerial parts of the *Pergularia daemia* were collected from the foot hills of Tirumala, Andhra Pradesh state and their identity was conformed at The Botanical Survey of India, Southern circle, Coimbatore, India. The voucher specimen (BSI/SC/5/21/05-06/Tech: 1512) was also deposited at the Madras herbarium, The Botanical Survey of India, Coimbatore.

2.2. Preparation of extracts

The shade dried aerial parts of about 500 g were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60–80 °C) and then extracted with 5 l of 95% ethyl alcohol using soxhlet apparatus till exhaustion for about 32 h. The total aqueous extract was also prepared by percolation method using 2.5 l of chloroform water till the percolate

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is colourless for about 30 h. Both the ethanolic and aqueous extracts were concentrated under vacuum to get the residues. The percentage yields of ethanolic extract and aqueous extract were found to be 3.9% (w/w) and 4.23% (w/w), respectively. The ethanolic extract was found to contain cardenolides, triterpenes and flavonoids (Wagner and Blatt, 1996). Silymarin was used as a positive control at an oral dose of 200 mg/kg (Morazzoni and Bombardelli, 1995). All the test suspensions are prepared in vehicle, i.e., Tween-80.

2.3. Animals

Wistar albino rats of either sex, weighing 200–250 g maintained under standard husbandry conditions (temperature 23 ± 2 °C, relative humidity 55 ± 10% and 12-h light:12-h dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, M.S. University of Baroda, Vadodara, Gujarat.

2.4. Toxicity studies

Acute toxicity study was performed for ethanolic and aqueous extracts according to the acute toxic classic method (as per OECD guidelines). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose, i.e., 2000 mg/kg. One-tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity, i.e., 200 mg/kg (Handa and Anupama, 1990).

2.5. Carbon tetrachloride-induced hepatotoxicity in rats

Rats were divided into five groups of six each, control, hepatotoxin, positive control and two test groups. The control group received oral vehicle treatment at 0, 24 and 48 h. The animals in hepatotoxin-treated group received vehicle at 0 h and at 24 h vehicle followed by carbon tetrachloride diluted in liquid paraffin (1:1, i.p.) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The test groups have received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of carbon tetrachloride and at 48 h the third dose of extracts (Kurma and Mishra, 1997; Sureshkumar and Mishra, 2005). The positive control group has received the first dose of silymarin (200 mg/kg) (Morazzoni and Bombardelli, 1995) at 0 h, at 24 h the second dose of silymarin followed by a dose of carbon tetrachloride and at 48 h the third dose of silymarin. After 72 h blood was collected from all the groups, and allowed to clot for the separation of serum. The serum was used for estimation of biochemical parameters. Glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) are estimated by Reitman and Frankel Method (1957), alkaline phosphatase (ALKP) by PNPP method (Mac Comb and Bowers, 1972), total bilirubin (TBL) by Jendrassik and Grof method (1938), total cholesterol (CHL) by CHOD-PAP Method (Richmond, 1973), total protein (TPTN) by colour complexation with copper ions in an alkali solution (Peters, 1968) and albumin (ALB) was estimated by Bromo Cresol Green Method (Webster, 1974). All the determinations were carried out using standard kits by an autoanalyser of Merck make (300 TX, E. Merck-Micro Labs, Mumbai).

2.6. Histopathological studies

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods (Galighor and Kozloff, 1976) and cut into 5 μm thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

2.7. Statistical analysis

The mean values ± S.E.M. are calculated for each parameter. For determining the significant inter-group difference each parameter was analysed separately and one-way analysis of variance (ANOVA) (Gennaro, 1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's Procedure (1964).

3. Results

The ethanolic and aqueous extracts did not cause any mortality up to 2000 mg/kg and were considered as safe (OECD, 1996). The rats which have received ethanolic extract at the dose of 2000 mg/mg exhibited ptosis.

Carbon tetrachloride (CCl_4) intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP, TBL and CHL, whereas decrease in the levels of TPTN and ALB were observed significantly indicating acute hepato cellular damage and biliary obstruction. The rats treated with ethanolic extract and also silymarin, showed a significant decrease in all the elevated SGOT, SGPT, ALKP, TBL and CHL levels and significant increase in TPTN and ALB levels (Table 1). The rats treated with aqueous extract have shown significant decrease in the levels of SGOT and CHL and increase in the levels of ALB.

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Fig. 1). Disarrangement of normal hepatic cells with intense centrilobular necrosis

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