Original Article

Role of plant metabolites in toxic liver injury

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Aphanamixis polystachya is a traditional medicinal plant of the Meliaceae family in India. A crude ethanolic extract of the leaf of this plant shows a beneficial effect on toxic liver injury. Its antihepatotoxic activity was evaluated on carbon tetrachloride (CCl_4)-induced liver injury in a rat model. The assessment of hepatoprotective activity was evaluated by measuring the activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH), serum total bilirubin and albumin and histology of the liver. The crude leaf extract significantly inhibits the enhanced ASAT, ALAT, ALP, ACP and LDH activities released from the CCl_4-intoxicated animals. It also ameliorated the depressed value of serum albumin and the enhanced value of total bilirubin in plasma caused by CCl_4 intoxication. The study showed that the crude ethanolic extract from *A. polystachya* leaves provided protection against acute carbon tetrachloride-induced liver damage.

Key words: antihepatotoxic activity, *Aphanamixis polystachya,* carbon tetrachloride, hepatoprotective, India, medicinal plants, Meliaceae plant family, phytotherapy.

Introduction

The liver is the key organ of metabolism and detoxification. Continuous exposure to a variety of environmental toxic agents enhances hepatic injury.[1] A growing interest has emerged around the globe in rediscovering medicinal plants as useful therapeutic agents for the prevention of such injury. Successful liver therapy owes much to the identification of pathogenesis and elaboration of suitable models of hepatic injury, comparable to those encountered in clinical practice.[2]

In the present study, a liver disease model was produced with a potent hepatotoxin, carbon tetrachloride (CCl_4), to evaluate the sequence of pathophysiological events and biochemical pharmacology. The Meliaceae plant family has long been used in India for its medicinal properties. *Aphanamixis*, a genus of this family, has been used in this study. The crude ethanolic stem bark extract of *Aphanamixis polystachya* has shown hepatoprotective activities.[3–7] In this investigation, crude ethanolic leaf extract has been used to determine its efficacy against liver damage, using the activities of marker enzymes such as plasma aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH), and the plasma concentrations of bilirubin and albumin.

Materials and methods

Male rats (Charles Foster strain, body weight 100–150 g) were procured from the animal house, Department of Physiology, University of Calcutta, and were kept in ventilated cages. Animals were acclimatised to laboratory conditions with a standard pellet diet (Hindustan Lever, India) and water ad libitum for 15 days.

A crude ethanolic leaf extract of the plant was used for the study and was prepared as follows. After identification of the plant from the Botanical Survey of India, Calcutta, fresh leaves of the plant were washed with water, dried in the shade and then ground into a powder, which was mixed with 90% ethanol. The mixture was allowed to stand for 20 days in a dark, cool place and then filtered. The alcohol extract of the plant was dried under reduced pressure at room temperature. It was suspended in distilled water with 0.25% nutrient agar. Fresh suspensions were prepared daily. Experimental liver damage was produced by injecting carbon tetrachloride intraperitoneally at a dose of 2 mL/kg (as a 30% solution in liquid paraffin).

Experimental design

Group I (Normal control). Rats were given a standard pellet diet (Hindustan Lever, India) and water ad libitum. They also received liquid paraffin (2 mL/kg body weight) intraperitoneally on 7 days and nutrient agar (1 mL of 0.25% nutrient agar/kg body weight) orally for 7 days, including injection day.

Group II (Experimental control). Rats were treated orally with 1 mL of 0.25% nutrient agar/kg body weight for 7 days. A single dose of CCl_4 (2 mL/kg body weight) as a

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30% solution in liquid paraffin was administered intraperitoneally on the seventh day.

Group III (Treated groups). Rats were treated with a crude ethanolic extract of *A. polystachya* leaf as a suspension in 1 mL of 0.25% nutrient agar. The dosage was 50 mg/kg body weight administered orally for 7 days. On the seventh day, CCl_4 (2 mL/kg body weight) as a 30% solution in liquid paraffin was administered intraperitoneally. After completion of treatment, all animals were fasted for 24 h prior to sacrifice. Blood was collected in a heparinised syringe by cardiac puncture, centrifuged and the supernatant plasma was used for biochemical analysis. Biochemical assessments were performed with plasma ALAT and ASAT,[8] ALP and ACP,[9] LDH,[10] total bilirubin and albumin.[11] A portion of the liver tissue was fixed in Bouin's fixative and stained with eosin–haematoxylin stain for histopathological studies by the standard method.

Analysis of results

Hepatoprotective activity was expressed as the percentage of protection. This was calculated following the method of Lexa *et al.*[12]

Results

The effects of the crude ethanolic leaf extract of *A. polystachya* on plasma transaminases, alkaline and acid phosphatase, lactate dehydrogenase activity and total serum bilirubin and albumin concentrations of rats intoxicated with CCl_4 are shown in Fig. 1. The levels of plasma enzymes and total bilirubin concentration elevated by CCl_4 in the experimental control group were lowered while the depressed albumin concentration of plasma was elevated by administration of the leaf extract. The percentages of protection were: ASAT, 75.432; ALAT, 88.667; ALP, 89.175; ACP, 81.264; LDH, 80.992; total bilirubin, 76.176; total albumin, 71.524. In the experimental control group, histological findings showed prominent macro- and microvesicular changes with well documented fat globules in the cytoplasm. Some of the sinusoids were open showing Kuppfer's cells. However, the test group (leaf extracts given for 7 days prior to CCl_4 injection) showed less fatty globules in the cytoplasm (Fig. 2).

Discussion

Elevation of the plasma levels of cytoplasmic and mitochondrial enzymes is a sensitive indicator of liver damage.[13] Drug-induced liver damage has been reported to correlate with an increase in the activity of these enzymes.[14] Among the various phosphatases, ACP and ALP have attained much attention because of their location in the plasma membrane and possible role in active transport.[15] Plasma levels of LDH also bear a close relationship to hepatocellular damage. The efficacy of any hepatoprotective drug is essentially dependent

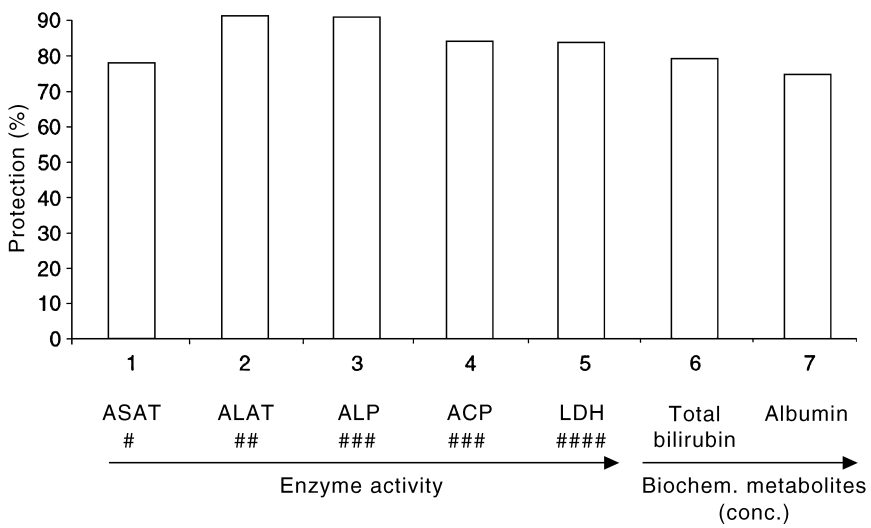

Figure 1. Percentage of protection from liver disorders by crude ethanolic *A. polystachya* leaf extract. ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ACP, acid phosphatase; LDH, lactate dehydrogenase; *#*, mg of oxaloacetate liberated/10 mg total protein/h at 37°C; *##*, mg of pyruvate liberated/10 mg total protein/h at 37°C; *###*, mg of p-nitrophenol liberated/10 mg total protein/30 min at 37°C; *####*, µmol/L of NADH oxidised/min/mL of plasma.

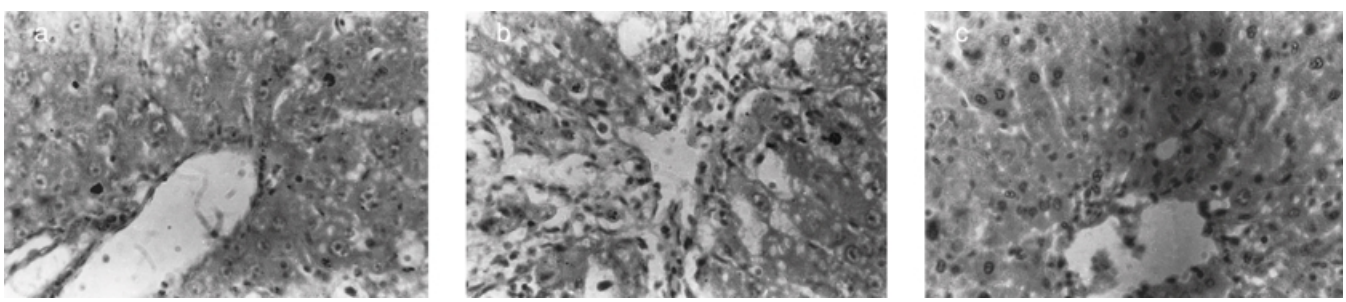

Figure 2. Photomicrograph showing the effects of the crude ethanolic leaf extract on (a) normal control liver (group I), (b) experimental control liver (group II) and (c) treated liver (group III).

on its capability of either reducing the harmful effects of a hepatotoxin or of maintaining the normal physiological mechanisms that are unbalanced by a hepatotoxin. The mechanism of CCl_4-induced liver lesions has been fairly well documented.[16] CCl_4 toxicity is responsible for the production of toxic trichloromethyl free radicals (CCl_3•) by the liver microsomes during the metabolism of CCl_4.[17]

In the present study, elevation of the plasma levels of cytoplasmic enzymes following CCl_4 intoxication accurately reflected damage to the liver by the accumulation of fat globules in the cytoplasm. When the ethanolic leaf extract was administered for 7 days prior to CCl_4-induced damage, there was a considerable reduction in the elevated enzyme levels caused by CCl_4 toxicity and in the accumulation of fat globules. Fat globule accumulation followed by subsequent tissue destruction is one of the mechanisms of CCl_4-induced damage.[18] The ameliorating effect may have been due to inhibition of CCl_4-induced bioactivation or it may be assumed that the initial stages of CCl_4-induced cell damage, leading to fatty accumulation, was prevented, or both. It may thus be concluded that the crude ethanolic leaf extract of *A. polystachya* showed a potent protective effect on carbon tetrachloride-induced acute toxic liver injury.

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