Hepatoprotective activity of hydroalcoholic extract of leaves of *Alocasia indica* (Linn.)

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Oral administration of hydroalcoholic extract of *A. indica* (250 and 500 mg/kg) effectively inhibited CCl4 and paracetamol induced changes in the serum marker enzymes, cholesterol, serum protein and albumin in a dose-dependent manner as compared to the normal and the standard drug silymarin-treated groups. Hepatic steatosis, fatty infiltration, hydropic degeneration and necrosis observed in CCl4 and paracetamol-treated groups were completely absent in histology of the liver sections of the animals treated with the extracts. The results suggests that the hydroalcoholic extract of leaves of *A. indica* possess significant potential as hepatoprotective agent.

Keywords: *Alocasia indica*, CCl4, Hepatoprotection, Paracetamol, Silymarin

Liver diseases such as jaundice, cirrhosis and fatty liver diseases are very common and large public health problem in the world1. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate2,3. There is no rational therapy available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine. The modern medicines have little to offer for alleviation of hepatic ailments whereas most important representatives are of Phytocconstituents4,6. The traditional system of medicine like Ayurveda and Siddha system of medicine, Unani system, Chinese system of medicine, Kampoo (Japanese) system of medicine have a major role in the treatment of liver ailments.

Some oriental medicinal plants are used in treatment of hepatobiliary pathologies7. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for treatment of liver diseases. However, there were not enough scientific investigations on the hepatoprotective activities conferred to these plants. One of such plant from Indian flora is *Alocasia indica* Linn. It is believed in folklore that the water decoction of the leaves of this plant can cure jaundice within seven days. Different parts of this plant are used in inflammation and in diseases of abdomen and spleen8. The juice of the leaves of the plant is used as digestive, laxative, diuretic, astringent and traditionally used for the treatment of rheumatic arthritis9. *Alocasia indica* has antifungal properties10. The plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, malic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines11. Since no scientific data are available to justify the traditional hepatoprotective potential of the plant, present study has been planned to validate the therapeutic use of *A. indica* in treatment of liver disorder.

Materials and Methods

**Chemicals**— Carbon tetrachloride used was of analytical grade and procured from E. Merck (India) Ltd. Mumbai; Paracetamol was procured from Torrent Research Centre, Ahmedabad. Silymarin was obtained as gift sample from M/s Micro Labs, Bangalore. Other solvents and chemicals were of analytical grade and purchased locally.

**Experimental animals**— Wistar albino rats weighing 175-225 g of either sex were obtained from Krishna Institute of Medical Sciences, Karad, Dist- Satara (Maharashtra) and were acclimatized for 10 days under standard housing conditions (24° ±1°C; 45-55% RH with 12:12 h light/dark cycle). The animals had free access to rat food (Lipton Gold Mohr, India) and water. The animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize any non-specific stress. The experimental

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protocol was approved by the Institutional Animal Ethics Committee by Government College of Pharmacy, India and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Plant material— Fresh leaves of Alocasia indica Linn. (Araceae) collected from different places at Karad were authenticated by Prof. S. K. Patil, Botany Dept., Yashwantrao Chavan College of Science, Karad and a voucher specimen was deposited at the Institute’s Herbarium.

Preparation of extract— Fresh leaves of A. indica were separated from plant and allowed to dry in sun light (30°C, 45% RH) for 15 days and then homogenized to get a coarse powder. Powder (250 g) was extracted with hydroalcoholic mixture (ethanol - 47.5% and water in 1:1 proportion) at room temperature by cold maceration method\(^\text{12}\). The filtrate was collected and concentrated on heating mantle at 45°C till a syrupy mass was obtained. The extract was again dried by rotary evaporator and kept under refrigeration. The percentage yield was found to be 2.56 with respect to the initial dried plant material. The hydroalcoholic extract of Alocasia indica was referred as AI.

Phytochemical screening — A preliminary phytochemical screening of hydroalcoholic extract of Alocasia indica was carried out\(^\text{13}\).

Drug formulations — Oral suspensions containing 250 and 500 mg/ml of hydroalcoholic extract of A. indica were prepared in gum tragacanth (1% w/v).

Acute toxicity study — Acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticide and Toxic Substances) Up and Down procedure\(^\text{14}\).

Evaluation of hepatoprotective activity — Hepatoprotective activity was evaluated using acute hepatic injury models induced by CCl\(_4\) and paracetamol.

CCl\(_4\) induced liver toxicity\(^\text{15}\) — The CCl\(_4\) was diluted with liquid paraffin (1:1) before intraperitoneal administration. The animals were divided into following 5 groups of 5 each.

Group 1: vehicle (50 % aqueous sucrose solution) for 9 days.

Group 2: vehicle + CCl\(_4\) (1 ml/kg) on ninth day.

Group 3: Silymarin (100 mg/kg/day, po) + CCl\(_4\) (1 ml/kg, po) on ninth day.

Group 4: AI (250 mg/kg/day, po) + CCl\(_4\) (1 ml/kg, po) on ninth day.

Group 5: AI (500 mg/kg/day, po) + CCl\(_4\) (1 ml/kg, po) on ninth day.

To enhance the acute liver damage in animals of groups 2, 3, 4 and 5, food was withdrawn 12 h before CCl\(_4\) administration. Animals were sacrificed 24 h after administration of CCl\(_4\). Blood samples were collected by puncturing the retro-orbital plexus under light ether anaesthesia and allowed to coagulate for 30 min at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters. The liver was removed after sacrifice and observed for weight, volume and appearance, washed with normal and then fixed in 10% formalin for histopathological studies.

Paracetamol (PCM) induced liver toxicity\(^\text{16}\) — The same procedure as mentioned above was followed except that the liver was damaged using PCM (1 g/kg, po) diluted with sucrose solution (40% w/v). PCM was administered intraperitoneally in 3 divided doses on 9\(^\text{th}\) day and animals were sacrificed 48 h after administration of PCM.

Assessment of liver functions — The hepatoprotective effect of extract was evaluated by the assay of liver function biochemical parameters such as alanine amino transferase (ALT)\(^\text{17}\), aspartate amino transferase (AST)\(^\text{17}\), alkaline phosphatase (ALP)\(^\text{18}\), total serum bilirubin (SB)\(^\text{19}\), total cholesterol (CHL)\(^\text{20}\), total protein (TP)\(^\text{21}\) and serum albumin (SA)\(^\text{22}\) according to standard methods.

Histopathological studies — The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was observed for weight (LW), volume (LV) and appearance. The liver was washed with normal saline and 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\(^\text{23}\) and cut into 5 µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were observed under compound microscope for histopathological changes in liver architecture and their microphotographs were taken.

Pentobarbitone sleeping time test\(^\text{24}\) — The rats were kept on standard diet. Experiments were set as mentioned above for CCl\(_4\) and paracetamol. After 24 h of the last treatment by drug formulations of AI in
CCl₄ model and 48 h after the last treatment by drug formulations of AI in paracetamol treatment, pentobarbitone sodium in water (35 mg/kg) was injected intraperitoneally. Food was withdrawn and water given ad libitum 12 h before pentobarbitone injection. All the experiments were conducted between 0900 to 1700 h in temperature controlled room. The animals were placed on table after loss of righting reflex. The time interval between loss and regain of righting reflex was measured as pentobarbitone sleeping time (PST). This functional parameter was used to determine the metabolic activity of the liver.

Statistical analysis — The statistical significance was assessed using one way of variance (ANOVA) followed by Bonferrini’s multiple comparison tests. The values are expressed as mean ± SE and P < 0.05 was considered significant.

Results

Preliminary phytochemical investigation — The preliminary phytochemical investigation of the hydroalcoholic extract of A. indica showed that it contains flavonoids, cynogenetic glycosides, citric acid, ascorbic acid, polyphenolic compounds.

Acute toxicity studies — Hydroalcoholic extracts of AI did not produce any mortality up to a dose of (2000 mg/kg, po). Hence 1/8th (250 mg/kg, po) and 1/4th (500 mg/kg, po) of this dose were employed for further pharmacological investigations.

CCl₄ and paracetamol (PCM) induced toxicity — CCl₄ and PCM intoxication in normal rats elevated the levels of serum marker enzymes ALT, AST, ALP, SB, CHL and decrease in the levels of TP, SA were observed significantly indicating acute hepatocellular damage and biliary obstruction. Pretreatment with AI produced a significant reduction in serum marker enzymes ALT, AST, ALP, SB, CHL and increase in the levels of TP, SA similar to Silymarin (100 mg/kg, po) treated group which seems to offer the protection and maintain the functional integrity of hepatic cells. Pretreatment with AI and Silymarin significantly reduced the increase in the liver weight and liver volume seen after CCl₄ and PCM intoxication. A significant reduction in thiopentone-induced sleeping time was observed with AI extract as compared to the CCl₄ and PCM treated group (Tables 1 and 2).

Histological examination of the liver tissue from CCl₄ and PCM treated animals revealed that CCl₄ and PCM had produced profound inflammation, severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus congestion especially in the sinusoids and occasional necrosis. Pre treatment of animals with Silymarin, AI (250 mg/kg, po) and AI (500 mg/kg, po) reduced the inflammation, degenerative changes and steatosis (Figs 1 and 2).

Discussion

Hydroalcoholic extract of A. indica used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided similar to that produced by Silymarin (100 mg/kg; po), a well known hepatoprotective agent. The hepatotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, po)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (mg/dl)</th>
<th>SB (mg/dl)</th>
<th>CHL (mg/dl)</th>
<th>TP (mg/dl)</th>
<th>SA (mg/dl)</th>
<th>LW (g/100g body wt.)</th>
<th>LV (ml)</th>
<th>PST (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>74.23±1</td>
<td>178.98±1</td>
<td>393.56±1</td>
<td>0.29±1</td>
<td>184.96±1</td>
<td>7.34±1</td>
<td>4.49±1</td>
<td>3.6±1</td>
<td>3.75±1</td>
<td>125.58±1</td>
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<tr>
<td>control</td>
<td>-</td>
<td>5.34</td>
<td>6.78</td>
<td>7.45</td>
<td>0.07</td>
<td>5.2</td>
<td>0.07**</td>
<td>0.04***</td>
<td>0.05</td>
<td>0.09</td>
<td>3.99***</td>
</tr>
<tr>
<td>CCl₄ control</td>
<td>-</td>
<td>381.23±1</td>
<td>650.56±1</td>
<td>745.23±1</td>
<td>1.48±1</td>
<td>394.54±1</td>
<td>3.84±1</td>
<td>2.15±1</td>
<td>3.64±1</td>
<td>3.97±1</td>
<td>200.88±1</td>
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<tr>
<td>CCl₄ control + Silymarin</td>
<td>100</td>
<td>56.12±1</td>
<td>211.78±1</td>
<td>418.06±1</td>
<td>0.32±1</td>
<td>196.79±1</td>
<td>6.72±1</td>
<td>4.15±1</td>
<td>3.6±1</td>
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<td>CCl₄ + AI</td>
<td>250</td>
<td>61.12±1</td>
<td>532.45±1</td>
<td>623.67±1</td>
<td>0.34±1</td>
<td>225.34±1</td>
<td>6.07±1</td>
<td>3.14±1</td>
<td>3.6±1</td>
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<td>139.30±1</td>
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<td>21.34±1</td>
<td>167.23±1</td>
<td>458.56±1</td>
<td>0.32±1</td>
<td>212.49±1</td>
<td>6.5±1</td>
<td>3.94±1</td>
<td>3.51±1</td>
<td>3.7±1</td>
<td>136.39±1</td>
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</tbody>
</table>

P : < 0.001 vs. vehicle control, b < 0.05 vs. vehicle control, m > 0.05, * < 0.05, ** < 0.01, *** < 0.001 vs. CCl₄ treated control
ALT = alanine amino transferase; AST = aspartate amino transferase; ALP = alkaline phosphatase; SB = total serum bilirubin; CHL = total cholesterol; TP = total protein; SA = serum albumin; LW = liver weight; LV = liver volume; B.W. = body weight; PST = pentobarbitone sleeping time

Table 1 — Effects of silymarin and hydroalcoholic extracts of A. indica (AI) on ALT, AST, ALP, SB, CHL, TP, SA, LW, LV and PST in CCl₄ induced liver toxicity in rats

[Values are mean ± SE from 5 animals in each group]
Table 2— Effects of silymarin and hydroalcoholic extracts of A. indica (AI) on ALT, AST, ALP, SB, CHL, TP, SA, LW, LV and PST in paracetamol (PCM) induced liver toxicity in rats
[Values are mean ± SE from 5 animals in each group]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (po)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (mg/dl)</th>
<th>SB (mg/dl)</th>
<th>CHL (U/L)</th>
<th>TP (mg/dl)</th>
<th>SA (mg/dl)</th>
<th>LW (g/100g body wt.)</th>
<th>LV (ml)</th>
<th>PST (min)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>73.33±</td>
<td>176.18±</td>
<td>394.16±</td>
<td>0.24±</td>
<td>186.46±</td>
<td>8.24±</td>
<td>4.29±</td>
<td>3.13±</td>
<td>3.65±</td>
<td>129.84±</td>
</tr>
<tr>
<td>PCM control</td>
<td>-</td>
<td>388.31±</td>
<td>652.46±</td>
<td>735.35±</td>
<td>1.38±</td>
<td>398.43±</td>
<td>3.94±</td>
<td>3.55±</td>
<td>4.97±</td>
<td>4.07±</td>
<td>207.88±</td>
</tr>
<tr>
<td>PCM + Silymarin</td>
<td>100</td>
<td>84.22±</td>
<td>172.68±</td>
<td>327.08±</td>
<td>0.28±</td>
<td>197.67±</td>
<td>6.98±</td>
<td>4.15±</td>
<td>3.15±</td>
<td>3.63±</td>
<td>141.31</td>
</tr>
<tr>
<td>PCM + AI 250</td>
<td>99.2±</td>
<td>185.65±</td>
<td>632.37±</td>
<td>0.47±</td>
<td>232.43±</td>
<td>6.76±</td>
<td>3.94±</td>
<td>3.20±</td>
<td>3.67±</td>
<td>149.35±</td>
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<tr>
<td>PCM + AI 500</td>
<td>89.36±</td>
<td>179.23±</td>
<td>342.6±</td>
<td>0.54±</td>
<td>219.39±</td>
<td>6.95±</td>
<td>4.12±</td>
<td>3.18±</td>
<td>3.77±</td>
<td>139.29±</td>
<td></td>
</tr>
</tbody>
</table>

P : * < 0.01 vs. vehicle control, ** < 0.05 vs. vehicle control, *** > 0.05, **** < 0.01, ***** < 0.001 vs. PCM treated control
ALT = alanine amino transferase; AST = aspartate amino transferase; ALP = alkaline phosphatase; SB= total serum bilirubin; CHL= total cholesterol; TP = total protein; SA = serum albumin; LW =liver weight; LV =liver volume; B.W. = body weight PST= pentobarbitone sleeping time

Fig. 1— Effect of hydroalcoholic extract of A. indica (AI) on acute liver injury induced by CCl₄ [A: (control): liver section with normal structure and architecture, B: (CCl₄ treated): showing extensive area of necrosis, profound inflammation and congestion, C: (CCl₄ +AI extract 250 mg): reduced inflammation, degenerative changes and steatosis, D: (CCl₄ +AI extract 500 mg): limited area of inflammation, necrosis, degenerative changes, and steatosis (H & E × 200)]
of CCl₄ has been reported to be due to the formation of the highly reactive trichloro (CCl₃) free radical, which alters functions of endoplasmic reticulum and causes peroxidative degradation of lipid membrane of the adipose tissue²⁶, leading to loss of metabolic enzymes located in the intracellular structures²⁷. It is known that PCM induces liver injury through the action of its toxic metabolite, N-acetyl-p-benzoquinoneimine, produced by the action Cytocrome P-450. This metabolite causes depletion of glutathione (GSH) leading to cell death²⁸, ²⁹.

It is evident that the AI extract was able to reduce all the elevated levels of AST, ALT, ALP, bilirubin and cholesterol towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by hepatotoxins. The raise in protein and albumin levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with AI extracts the normal cellular architecture was retained as similar to silymarin treated rats, there by confirming the protective effect of the extracts. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume³⁰ as observed in the present study. This hepatotoxin-induced increase in total wet-liver weight and volume were prevented by pretreatment with AI extracts, thus indicating a hepatoprotective effect. Pre-treatment with hydroalcoholic extract of A. indica decreased thiopentone-induced sleep time, an indirect evidence of their hepatoprotective effect.

AI contains chief constituent alocasin, flavonoids and glycosides¹¹. The flavonoid constituents possess free radical scavenging properties³¹. Pretreatment with AI showed a dose-dependent protection against the injurious effects of CCl₄ and PCM that may result from the interference with cytochrome P₄₅₀, resulting in the hindrance of the formation of hepatotoxic free radicals. It can be concluded that hydroalcoholic extract of A. indica possess a protective effect against...
CCl₄ and PCM-induced hepatotoxicity in rats, as evidenced by the physical, biochemical, functional and histological parameters. Hepatoprotective potential of hydroalcoholic extract of A. indica may be due to presence of alocasin, flavonoids and other polyphenolic moieties present in it.

The isolation and testing of constituents likely to be responsible for the hepatoprotective activity of A. indica and evaluation of the exact mechanism of action is under progress in our lab.

Acknowledgement

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References