HEPATO-PROTECTIVE VALUE OF SOME PLANTS EXTRACT AGAINST CARBON TETRACHLORIDE TOXICITY IN MALE RATS

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Abstract: Carbon tetrachloride (CCl₄) is well known for its hepatotoxicity in human and experimental animals. One of the main mechanisms attributed to CCl₄ induced hepatotoxic effects is generation of reactive oxygen species / oxidative stress. Various plant extracts have been shown to possess significant hepato-protective properties. The present study plans to evaluate the protective efficacy of aqueous extracts of few common Indian medicinal plants like Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos, against CCl₄ induced hepatotoxicity in rats. Animals were exposed for 15 days through gastric intubation to CCl₄ (1ml/kg, 30% (v/v) with paraffin oil) followed by aqueous extract of Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos (2ml/kg body weight, 22.22% w/v) for 15 days. Biochemical variables indicative of liver function and oxidative stress were measured. Animals exposed to CCl₄ showed significant increase in serum glutamic oxaloacetic and glutamic pyruvic transaminase (AST and ALT) activities; serum alkaline phosphatase (ALP) activity and total bilirubin level accompanied by significant decrease in total cholesterol, albumin and protein levels. Hepatic thiobarbituric acid reactive substances (TBARS) level exhibited an increase while there was a significant depletion in catalase (CAT) and reduced glutathione (GSH) levels following exposure to CCl₄. Co-administration of aqueous plant extracts of Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum or Aegle marmelos with CCl₄ provided significant protection to most of the above mentioned biochemical variables, Maytenus emerginata being the best among all the extracts examined. The results led us to conclude the protective value of Maytenus emerginata in protecting animals from the hepatotoxic effects of CCl₄.

Key words: Hepato-protection, Medicinal plants, Carbon tetrachloride

INTRODUCTION

Chemical induced liver injury is known as toxic hepatopathy and one of the most common and important environmental health and clinical problem world wide. The liver is often abused by environmental toxicants, poor eating habits and over the counter drug use, which can damage the liver and eventually leads to hepatitis, cirrhosis and liver diseases.

Carbon tetrachloride is one of the xenobiotic, known for its severe hepatotoxic effects in humans and in animals. Over production of free radicals are toxic to hepatocytes and initiate reactive oxygen species (ROS) mediated cascade causing hepatocyte death leading to acute hepatic damage [1,2]. Reductive dehalogenation of CCl₄ resulted in the generation of trichloromethyl and trichloromethyl peroxy free radicals responsible for cellular oxidative stress; leading ultimately to liver damage [3]. The pharmaceutic imbalance between remedies that protect the liver and have antioxidant capacity and drugs that induced hepatotoxicity has promoted and accelerates search into plants used in folk medicine to treat liver diseases and increases liver functions [4].
In present study anti-hepatotoxic and antioxidant potential of above mentioned plant extracts against CCl₄ toxicity have been compared in rats based on certain sensitive biochemical parameters.

**MATERIALS AND METHODS**

**Chemicals and Reagents:** All the chemicals and reagents were procured commercially from Sigma Chemicals (USA), Merck (Germany), Fluka (Germany) or SD-Fine (India). Plants were collected from Anand Agriculture University (India). The plant extracts were stored in refrigerator in desiccators to avoid oxidation and microbial growth.

**Plant extract preparation:** Phyllanthus niruri, Maytenus emarginata, Eclipta alba, Aloe Vera, Solanum indicum or Agle Marmelos leaves were grinded to prepare 22.22 g % (w/v) crude homogenate, filtered through muslin cloth, refiltered using Whatman’s filter paper (No. 100). The aqous filtrate was used as plant extract for treating animals.

**Animals:** Male albino rats weighing 200±20g were used in the present study. Prior to use they were acclimatized for 7 days to dark and light cycle (12h/12h) at 25°C. They were housed (3 per cage) in clean polypropylene cages (38 x 23 x 10 cm) and maintained at standard laboratory condition. Animals were divided into eight groups of six rats each and treated orally as below for 15 days.

**Group-I:** Control receiving carrier.

**Group-II:** CCl₄ 1 ml/kg body weight (30 % (v/v) in paraffin oil)

**Group-III:** CCl₄ as in group-II followed by Phyllanthus niruri extract 2ml/kg body weight (22.22% (w/v) in distilled water)

**Group-IV:** CCl₄ as group-II followed by Maytenus emerginata extract 2ml/kg body weight (22.22% (w/v) in distilled water)

**Group-V:** CCl₄ as group-II followed by Eclipta alba extract 2ml/kg body weight (22.22% (w/v) in distilled water)

**Group-VI:** CCl₄ as group-II paraffin oil followed by Aloe vera extract 2ml/kg body weight (22.22% (w/v) in distilled water)

**Group-VII:** CCl₄ as group-II followed by Solanum indicum extract 2ml/kg body weight (22.22% (w/v) in distilled water)
Group-VIII: CCl₄ as group-II followed by *Aegle marmelos* extract 2ml/kg body weight (22.22% (w/v) in distilled water).

All animals received human care in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animal ethical committee of S. P. University, Vallabh Vidyanagar, India approved the protocol of the experiments. Animals were kept on standard pellet diet (Amrut Laboratory, Pranav Agro Industries Ltd. Vadodara) and water *ad libitum*.

Animals were sacrificed under ether anesthesia 24-h after the last dose. Animals were fasted overnight before sacrifices. Blood was collected in plain tube and liver was removed, rinsed in cold saline, soaked in Whatman filter paper, weighed and used for further biochemical analysis.

Sample preparation and the assay of biochemical markers: Serum was separated by centrifugation at 3000rpm at 4 °C for 10 min and used for various biochemical assays like glutamic oxaloacetic and glutamic pyruvic transaminase (AST and ALT) activities, serum alkaline phosphatase (ALP) activity, total bilirubin, total cholesterol, total albumin using commercially available kits (Crest Bio Systems, A division of Coral Clinical Systems, Verna, Goa-403 722, India) whereas, serum total protein was measured according to the method of Lowry et al. [16]. A 10% (w/v) liver homogenate was prepared in 0.25M sucrose solution and centrifuged at 700g at 4°C for 10 min. Supernatant was used for the estimation of thiobarbituric acid reactive substances (TBARS), reduced glutathione content and catalase activity.

**Glutathione content:** 200 μl of liver homogenate was added into 0.8ml of distilled water and 0.5ml of 10% sulphosalicylic acid, mixture was vortexed and centrifuged at 3000 rpm for 10 min. 0.5ml of supernatant was added in 4.5ml of 0.5M, pH8.23 tris-phosphate buffer and 0.5ml of dithiobis-2-nitrobenzoic acid. O.D. of the samples was read at 420 nm [17].

**Assay of catalase:** 0.1ml of liver homogenate was added in 1ml of 0.01M phosphate buffer (pH 7.1), 0.5ml distilled water and incubate at 37°C for 10 min. 0.4ml of 0.2M H₂O₂ was added in incubated tubes at 37 °C, 2.0ml of 5% potassium dichromate (1:3 with glacial acetic acid) was added at interval of 30s in both control and experimental tubes. All the tubes were incubated in boiling water bath for 15 min. Tubes were centrifuged at 5000 rpm for 15 min to remove precipitation. Supernatant was used for estimation of catalase activity at 570 nm [18].

**Lipid peroxidation:** 0.1 ml of liver homogenate was added in 0.9 ml of distilled water and 2.0 ml of TBA-TCA solution, incubated in boiling water bath for 15 min, cooled and centrifuged at 5000 rpm for 20 minutes. Supernatant was collected and estimated for MDA at 535 nm [19].

**Statistical analysis:** Statistical analysis was performed using SPSS version 10.0 and microsoft excel computer programs. Data comparisons were carried out using one-way analysis of variance (ANOVA) to compare arithmetic mean values between CCl₄ intoxicated, plants extract treated and control groups. Linear regression model were used to identify potential association between the variable. Statistical significance was accepted at the 95% confidence (p<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Total albumin (g/dl)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.56±4.46</td>
<td>0.15±0.13</td>
<td>4.87±0.11</td>
<td>8.3±1.4</td>
</tr>
<tr>
<td>CCl₄</td>
<td>22.94±3.74**</td>
<td>2.89±0.64**</td>
<td>2.3±0.19**</td>
<td>4.4±0.36**</td>
</tr>
<tr>
<td>CCl₄+P1</td>
<td>35.58±3.62†</td>
<td>1.27±0.4†</td>
<td>3.37±0.67†</td>
<td>6.5±0.53†</td>
</tr>
<tr>
<td>CCl₄+P2</td>
<td>39.75±3.16††</td>
<td>1.13±0.15††</td>
<td>3.42±0.66††</td>
<td>7.2±0.72††</td>
</tr>
<tr>
<td>CCl₄+P3</td>
<td>28.30±2.15†</td>
<td>1.22±0.62†</td>
<td>3.28±1.07†</td>
<td>6.1±0.23†</td>
</tr>
<tr>
<td>CCl₄+P4</td>
<td>25.31±2.67†</td>
<td>1.25±0.9†</td>
<td>3.05±0.6†</td>
<td>5.8±0.87†</td>
</tr>
<tr>
<td>CCl₄+P5</td>
<td>27.78±2.29†</td>
<td>1.54±0.21†</td>
<td>3.2±0.64†</td>
<td>6.5±0.32†</td>
</tr>
<tr>
<td>CCl₄+P6</td>
<td>30.73±1.97†</td>
<td>1.27±0.62†</td>
<td>3.42±1.07†</td>
<td>5.8±0.9†</td>
</tr>
</tbody>
</table>

Table 1: Effect of *Phyllanthus niruri* (P1), *Maytenus emerginata* (P2), *Eclipta alba* (P3), *Aloe vera* (P4), *Solanum indicum* (P5) and *Aegle marmelos* (P6) extract on cholesterol, total bilirubin, total albumin and total protein in CCl₄ induced hepatotoxicity in male Albino wistar rats. Values are means ± S.D.*p<0.05 compared to control group, **p<0.01 compared to CCl₄ treated group, †p<0.05 compared to control group, ††p<0.01 compared to CCl₄ treated group.
Fig. 1: Hepatotoxic marker enzymes (ALT, AST AND ALP) activity in control, CCl$_4$ and Phyllanthus niruri (P1), Maytenus emerginata (P2), Eclipta alba (P3), Aloe vera (P4), Solanum indicum (P5) and Aegle marmelos (P6) extract treated groups. *p<0.05 compared to control group, **p<0.01 compared to control group, †p<0.05 compared to CCl$_4$ treated group. Values are mean ± S.D.

Fig. 2: Enzymatic and Nonenzymatic Antioxidant (Catalase and Glutathione) status in control, CCl$_4$, Phyllanthus niruri (P1), Maytenus emerginata (P2), Eclipta alba (P3), Aloe vera (P4), Solanum indicum (P5) and Aegle marmelos (P6) extract treated groups. *p<0.05 compared to control group, **p<0.01 compared to control group, †p<0.05 compared to CCl$_4$ treated group, ††p<0.01 compared to CCl$_4$ treated group. Values are mean ± S.D.
RESULTS

Effect of plant extracts on serum enzymes, cholesterol, total bilirubin, total albumin and total protein: Activities of ALT, AST, ALP and bilirubin level showed a significant increase (Fig. 1) on CCl₄ exposure accompanied by a significant decrease in total cholesterol, total albumin and total protein levels (Table 1). Co-administration of Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos plant extracts with CCl₄ provided significant protection to the changes in serum transaminases, ALP activities and bilirubin level. Phyllanthus niruri and Maytenus emerginata provided more pronounced beneficial effects compared to other plant extract. Significant increase in total bilirubin level and depletion in albumin on CCl₄ response favorably to the co-administration of Phyllanthus niruri and Maytenus emerginata and Aegle marmelos providing better protection than other extracts examined in the present study. Decreased total serum protein concentration on CCl₄ exposure also showed maximum protection on Maytenus emerginata co-administration.

CAT activity in liver tissues: The effect of various extracts on liver CAT activity is shown in Figure 2. Exposure to CCl₄ led to a significant depletion of CAT activity compared to the controls, while all the plants extracts were effective in preventing the inhibition when administered concomitantly with CCl₄. Maytenus emerginata providing the best effects compared to all other plant extract while Aloe vera extract was the least effective.

Glutathione level in liver tissue: Hepatic GSH level decreased significantly on CCl₄ administration compared to control (Fig. 2). CCl₄ induced depleted GSH level showed significant protection in animals concomitantly administered Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos extract. Maytenus emerginata extract treated group showed maximum protection compared to all other extracts.

MDA level: A significant increase in TBARS level was noted on CCl₄ exposure in liver compared to normal animals (Fig. 3). Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos were able to prevent induction of lipid peroxidation by significantly depleting TBARS formation. Phyllanthus niruri extract proved to be the best among all the other extracts in protecting liver from peroxidative injury (decreasing TBARS contents). Aloe vera extract on the other has also showed only marginal protection but the effects were less marked compared to Phyllanthus niruri treated group (54 ± 1.37).

Fig. 3: TBARS level in control, CCl₄, Phyllanthus niruri (P1), Maytenus emerginata (P2), Eclipta alba (P3), Aloe vera (P4), Solanum indicum (P5) and Aegle marmelos (P6) extract treated groups. *p<0.05 compared to control group, †p<0.05 compared to CCl₄ treated group, ††p<0.01 compared to CCl₄ treated group. Values are mean ± S.D.
DISCUSSION

Carbon tetrachloride is an occupational toxicant widely used as a solvent in insecticide industries and is correlated with high incidence of certain types of cancer [20]. Exposure to carbon tetrachloride (CCl₄) in humans and in animals is well known to cause severe hepatotoxicity [21]. The hepatocellular damage may be identified by significant increase in transaminases activity (ALT and AST). Plasma ALT and AST activity represents standard tests for hepatocellular integrity [22]. These measurements assess leakage of hepatic enzymes into the blood stream. We also observed significant increase in the activities of these enzymes which are in agreement with the similar results reported by Ahmed et al. [23] and Amer et al [21]. AST is predominantly found in mitochondria of hepatocytes and gets significantly increased during chronic hepatitis and cirrhosis; ALT mainly increased in patients having hepatitis, cirrhosis and obstructive jaundice. We observed significant protective value of *Phyllanthus niruri*, *Eclipta alba*, *Aloe vera*, *Solanum indicum* and *Aegle marmelos* extracts down regulates CCl₄ induced increased AST and ALT activities. The maximum protection was exhibited by *Maytenus emerginata* than other plants extract. The level of serum ALP is associated with cholestatis, bile salts and to a less extent with liver cell damage [24]. CCl₄ treated animals showed elevated serum ALP accompanied by a decrease in liver ALP activity, suggesting leakage of this enzyme into serum. The alteration in serum ALP on CCl₄ exposure showed significant protection on co-administration with *Maytenus emerginata*, *Eclipta alba*, *Aloe vera*, *Solanum indicum* and *Aegle marmelos*. *Phyllanthus niruri* exhibits the maximum protective value. The results based on ALP, AST and ALT activities in liver and serum clearly establish the excellent hepatoprotective properties of *Phyllanthus niruri* and *Maytenus emerginata* compared to other examined plants extracts.

CCl₄ exposed animals showed decrease in serum total protein and albumin contents. Albumin is the most abundant plasma protein produced by hepatocytes; its depreciation usually reflects decreased hepatic synthesis. This fall is often attributed to decrease hepatic function of albumin synthesis. Decreased total albumin and total protein may also be due to kidney damage leading to the release of albumin with the urine. *Phyllanthus niruri*, *Eclipta alba*, *Aloe Vera*, *Solanum indicum* and *Aegle marmelos* restored hepatic protein synthesis and hepatocytes regeneration while, *Maytenus emerginata* extract showing the most pronounced effectiveness. The decrease in total cholesterol level can be attributed to the accumulation of triglycerides in the hepatic cells. An obstruction of the secretion of hepatic triglycerides into plasma is basic mechanism underlining the fatty liver induced in rat by CCl₄ [25]. *Phyllanthus niruri*, *Maytenus emerginata*, *Eclipta alba*, *Aloe vera*, *Solanum indicum* and *Aegle marmelos* restored the cholesterol concentration significant while, *Maytenus emerginata* showing the maximum protection. *Maytenus emerginata* might reduce the mitochondrial damage in hepatocytes and destroy the blockage for excretion of triglycerides. We also observed protective effects of *Phyllanthus niruri*, *Eclipta alba*, *Aloe vera*, *Solanum indicum* and *Aegle marmelos* in decreasing the serum total bilirubin in CCl₄ intoxicated animals which reflect the regeneration of liver cells, increases the capacity to take up circulating bilirubin and regulating the bilirubin conjugation. CAT is a key component of the antioxidative defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage.

Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Protective value of *Phyllanthus niruri*, *Eclipta alba*, *Aloe vera* against CAT activity has been reported earlier which has been attributed to its antioxidant property [6,10]. Increased lipid peroxidation in CCl₄ intoxicated group showed imbalance in redox status of liver [9,21]. Rapid and extensive lipid peroxidation of the membrane lipids has been proposed as a basis of CCl₄ hepatotoxicity. CCl₄ causes free radical induced oxidative damage to cells and causes lipid peroxidation, these processes may respond parallel to the generation of free radicals. Under some condition, lipid peroxidation may determine the extent of injury by amplifying the injury through propagation of free radical processes, generating toxic compounds and impairing detoxification systems [26]. CCl₄ treated group showed elevated level of lipid peroxidation.

Decrease in liver GSH content and catalase activity on CCl₄ exposure suggest decreased detoxification
Reduced glutathione (GSH) is an important constituent of cellular protective mechanisms against a number of toxic stimuli including oxygen derived free radicals [27]. The observed decrease in GSH level in the CCl₄ intoxicated animals due to an increased scavenging of reactive substances [21].

Our study showed that the supplementation of Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos regulate the lipid peroxidation by altering the liver GSH and antioxidant enzymes activities. The increased levels of GSH after Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos treatment may be attributed to an increased rate of GSH synthesis or due to increased uptake of extracellular GSH by hepatic tissue.

**CONCLUSION**

It can be concluded from the results that (i) the plant extracts of Phyllanthus niruri, Maytenus emerginata, Eclipta alba, Aloe vera, Solanum indicum and Aegle marmelos have significant protective value against CCl₄ induced hepatotoxicity and these values can partially be attributed to antioxidant free radical scavenging properties of these extracts and (ii) among the plants extract examined Maytenus emerginata showed the most pronounced effect than other plants studied. These results certainly show some future clinical importance, therefore, we recommend more studies with these plants extract, particularly Maytenus emerginata, to characterize the active principle ingredient(s) and to elucidate the possible mode of action. The work in this direction is in process.

**REFERENCES**