Original article

Effect of Careya arborea Roxb. on CCl₄ induced liver damage in rats

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Abstract

The aqueous extract of bark of Careya arborea Roxb., locally known as Kumbhi, was screened for hepatoprotective activity against CCl₄-induced hepatotoxicity in rats with a view to explore its application for treatment of liver disorders in animals and human beings. The hepatotoxicity induced by administration of carbon tetrachloride (CCl₄) as 30 % solution prepared in liquid paraffin and administered subcutaneously 1ml/kg b.wt. at every 72 h interval till the completion of experiment. The hepatotoxicity was found to be tolerated by simultaneous oral administration of aqueous extract of C. arborea (AECA) stem bark (100, 200 mg/kg b. wt.) for two weeks, with evidence of decreased level of AST, ALT, ALP and bilirubin. In addition, severe histomorphological disruption and fatty changes produced by CCl₄ in respect of cytoarchitecture were minimized and maintained by the treatment of extract. The results were compared with standard drug silymarine. The results of this study showed that AECA could afford hepatoprotective activity against CCl₄ induced liver damage in rats due to nutraceutical nature of plant.

Key words: Careya arborea Roxb., silymarine, hepatoprotective, carbon tetrachloride (CCl₄)

1. Introduction

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor (Wendel et al., 1987). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and the other oxidative damages (Dianzani et al., 1991).

Exposure of animals to the environmental pollutants, insecticides, pesticides, mycotoxins, toxic plants and synthetic drugs such as anticancer, corticosteroids, antibiotics result in the hepatic dysfunction and damage (Michael, 1984; Gupta and Solunkhe, 1985). Despite, enormous development in synthetic drugs, there is hardly any drug found to be absolutely safe to cure diseases of hepatic origin. Many herbs and herbal preparations were reported to possess the antioxidant and hepatoprotective properties. Herbal drugs have been used in the treatment of liver diseases for long time. C. arborea commonly known as “Kumbhi” in Hindi, “Kumbha” in Marathi and wild guava in English belongs to the family Lecythidaceae, is a medium sized deciduous tree, bark dark grey exfoliating in thin strip. The plant is widely available in India, Ceylon, Malay and Peninsula. The plant has been extensively investigated and chemical constituents from the barks, leaves and seeds of the plant, have previously been reported to include triterpenoids (Das and Manato, 1982), flavonoid (Gupta et al., 1975), cumarin (Basak et al., 1976), saponins and tannins (Kulakkattolickal, 1987). C. arborea have been used as folk medicine for centuries in India for its wide spectrum of pharmacological activities such as cytoxic, analgesic, anti-inflammatory, antioxidant and anticancer (Khaliq, 2016) and antiulcer activity (Kumar et al. 2013). It is also used as remedy for diarrhoea, dysentery with bloody stools and ear pain. Antipyretic, leech repellant, fish poison and antiinvenin activities have also been reported in literature (Kirtikar and Basu, 1975).

2. Materials and Methods

2.1 Plant material collection and identification

Plant C. arborea stem bark was collected from Shivangaon village near Nagpur city. The plant material was taxonomically identified from Department of Botany, RTM Nagpur University, Nagpur (Voucher Number-IS/RTM/345). The collected bark was dried in shade under room temperature and powdered.

2.2 Aqueous extract

One hundred grams of bark powdered material was taken in flask to which 2000 ml of distilled water was added. Then flask was kept on heating mantle for boiling at 100°C. Heating was done till the contents were reduced to one third of total content. The contents were cooled and filtered through muslin cloth so as to remove the insoluble material. The filter was again filtered through Whatman No. 42 filter paper and then the content was transferred to a dry, clean and already weighed petridish and placed on hotplate for complete evaporation. Care was taken to avoid charring. Then the extract was cooled at room temperature and again weighed to calculate the extractability percentage and finally stored in desiccator in cool and dry place.

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2.3 Phytochemical screening

A preliminary phytochemical screening of *C. arborea* was carried out according to the method of Wagner and Fintelmann (1999). The presence of alkaloids (Dragendorff reagent and mayer’s reagent), flavonoids (Shinoda test), steroids (Liberman Buchard test), Triterpenes (Vanillin sulfuric acid reagent), proteins (Xanthoprotein test), reducing sugar (Benedict’s reagent) and saponins (Foam test) were analyzed.

2.4 Experimental animals

The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol (F. No. 740-89-105/IAEC/2012). The present research work was carried out on thirty rats of albino Wistar strain, which were procured from the recognized Laboratory Animal Breeding Centre. Rats weighing around 150-180 g were used for the present study. All the animals were housed in polypropylene cages, (47×34×18 cm) lined with rice husk as a bedding material. They were provided with 12 h. light and 12 h. dark cycle. Animals were provided with *ad lib.* feed and wholesome drinking water throughout the period of experiment and acclimatized to laboratory condition for ten days before commencement of experiment.

2.5 Experimental protocol

The animals were randomly divided into five groups, each containing 6 animals and were dosed as below:

1st healthy control group: Received the 0.93% concentration normal saline (2ml/kg b.wt.) orally.

2nd positive control group: Received CCl₄ as 30% solution prepared in liquid paraffin and administered subcutaneously at dose rate of 1ml/kg b. wt. at every 72 h. interval for two weeks (Nagano et al., 2007).

3rd standard drug group: Received silymarin 25 mg/kg b. wt. orally for 2 weeks and simultaneously administered CCl₄ (1ml/kg b. wt.) at every 72 h.

4th treatment group: Received aqueous extract of *C. arborea* (AECA)100 mg/kg b. wt. orally for 2 weeks and simultaneously administered CCl₄ (1ml/kg b. wt.) at every 72 h.

5th treatment group: Received aqueous extract of *C. arborea* (AECA) 200 mg/kg b. wt. orally for 2 weeks and simultaneously administered CCl₄ (1ml/kg b. wt.) at every 72 h.

Study was carried out for two weeks and all the groups of the animals were sacrificed at end of the study.

2.6 Collection of samples

Blood was collected from orbital plexus of anaesthetized rats for hematology and serum biochemical analysis. The collected blood samples in 1% EDTA were subjected to hematological studies such as hemoglobin concentration (Hb) using Sahli’s method and total leucocyte count (TLC), total erythrocyte count (TEC) and packed cell volume (PCV) by method mentioned by Benjamin (1985). Blood samples from each rat were also collected for serum biochemical analysis. The separated serum was stored at −20°C for subsequent analysis. The biochemical tests with collected serum were performed on semi-automatic analyzer, using commercial biochemical kits (Span Diagnostics Ltd, Mumbai). Serum alanine amino transferase (ALT) and aspartate amino transferase were measured by method of Reitman and Frankel (Reitman and Frankel, 1957). Serum alkaline phosphatase activity was estimate according to method of Kind and King (Kind and King, 1954). Total serum bilirubin was estimated based on Diazoo method as per the method described by Henry (Henry, 1974).

**Figures A-E:** A. Photomicrograph of liver showing normal anatomical pattern of hepatocytes in section from group 1, B. Photomicrograph of liver showing fatty degeneration changes in perilobular area in section from group 2, C. Photomicrograph of liver showing fatty degeneration changes (fat droplets) in perilobular area and mild granulation, D. Photomicrograph of liver treated with AECA100 mg/kg b.w showing mild granular and vacuolar degenerative changes along with leukocyte infiltration. The central vein and portal triads appear normal in section from group 4, E. Photomicrograph of liver treated with AECA 200 mg/kg b.w. showing focal degenerative changes in along with cytoplasmic vacuolation of hepatocytes. Some of hepatocytes show binucleation suggests regenerative activity in the section from group 5.
2.7 Histopathological examination

The collected and weighed tissues were washed with normal saline and immersed and fixed in 10% buffered formalin immediately. The tissues were gradually dehydrated, embedded in paraffin, cut into 5µm sections and stained with hematoxylin and eosin for histopathological examination according to standard procedure described by Ross et al. (1989).

2.8 Statistical analysis

All the values in the test are presented as Mean ± SEM. Statistical differences between the means of the various groups were evaluated using completely randomized design. 'p' of less than 5% was considered to be statistically significant (p ≤ 0.05).

3. Results and Discussion

3.1 Phytochemical and safety studies

Preliminary phytochemical studies revealed the presence of alkaloids, steroids, saponins, proteins, reducing sugars, triterpenes and flavonoids. Findings in present phytochemical study were in agreement with the investigation of various researchers, in which Behera et al. (2012) reported the presence of phytoconstituents like tannins, flavonoids, terpenoids, cardiac glycosides, saponins, anthraquinones, sterols and phytosterols on primary phytochemical analysis using various solvents. Gupta et al. (2012) investigated presence of triterpenoids, saponins, tannins and flavonoids from stem bark. For acute oral toxicity studies, extract treated animals were observed for mortality up to 72 h. Based on the results, the treatment by extract was not produced any mortality up to 2000 mg/kg body weight in rats (Palanivel et al., 2008).

3.2 Liver weight

The mean organ weight of liver of each rat was studied and presented in Table 1.

3.3 Haemogram

The values of hemoglobin, total erythrocyte count (TEC), total leucocyte count (TLC) and packed cell volume (PCV) were significantly decreased in CCl₄ intoxicated group 2 when compared to group 1. On statistical analysis, the TLC and TEC values in treatment groups were significantly increased (p ≤ 0.05) when compared with group 2. However, Hb and PCV values were not significantly affected by the presence of extract in treatment groups. The hepatotoxicity induced by CCl₄, in respect to decreased concentration of Hb, TEC, TLC and PCV was found to be restored at some extent in extract treated groups.

Table 1: Effect of aqueous extract of C. arborea on haemogram, biochemical parameters and liver weight in experimental groups

<table>
<thead>
<tr>
<th>Parameter/Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.503±0.237</td>
<td>11.993±0.358</td>
<td>12.333±0.367</td>
<td>12.745±0.626</td>
<td>12.453±0.514</td>
</tr>
<tr>
<td>TEC (10⁶/mm³)</td>
<td>8.053±0.105</td>
<td>7.08±0.274</td>
<td>8.196±0.118</td>
<td>8.113±0.068</td>
<td>8.13±0.093</td>
</tr>
<tr>
<td>TLC (10⁶/mm³)</td>
<td>12.756±0.631</td>
<td>7.033±0.270</td>
<td>10.603±0.868</td>
<td>10.623±0.861</td>
<td>10.455±0.941</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.416±1.559</td>
<td>33.396±0.303</td>
<td>36.725±0.642</td>
<td>36.15±0.849</td>
<td>36.663±0.965</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>61.596±0.933</td>
<td>184.573±4.942</td>
<td>65.52±1.671</td>
<td>65.52±1.671</td>
<td>75.186±1.112</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>42.613±1.261</td>
<td>127.326±0.826</td>
<td>52.076±1.839</td>
<td>68.613±1.126</td>
<td>58.496±1.369</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>65.535±1.613</td>
<td>128.233±1.941</td>
<td>69.125±1.513</td>
<td>74.926±4.460</td>
<td>79.853±1.435</td>
</tr>
<tr>
<td>Bilirubin (IU/l)</td>
<td>0.86±0.03</td>
<td>2.673±0.062</td>
<td>0.883±0.017</td>
<td>1.250±0.008</td>
<td>1.125±0.027</td>
</tr>
<tr>
<td>Liver weight (gms)</td>
<td>5.596±0.338</td>
<td>5.353±0.440</td>
<td>6.063±0.471</td>
<td>5.205±0.339</td>
<td>4.776±0.264</td>
</tr>
</tbody>
</table>

Mean values carrying different superscripts a, b, c, d .... in columns differ significantly (p ≤ 0.05). Keys: 1-Healthy control, 2-Positive control, 3-Standard drug, 4-AECA 100 mg/kg b.w., 5-AECA 200 mg/kg b.w., TEC-total erythrocyte count, TLC-total leucocyte count, PCV-Packed cell volume, AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase. (n=6)

3.4 Serobiochemical changes

The activities of serum AST, ALT, ALP and serum bilirubin concentration increased significantly (p < 0.05) after CCl₄ administration as reported earlier (Nagano et al., 2007; Reddy et al., 2010; Elhag et al., 2011). In treatment groups (groups 3 and 4), these values decreased in a dose dependent manner, indicating less damage to the liver (Table 1). In group 3, treated with silymarin (25 mg/kg b.w) also significantly decreased level of serum AST, ALT, ALP and bilirubin concentration values as compared to respective group 2. After treatment with C. arborea, a significant restoration of these enzymes level by administration of aqueous extracts of plant at different doses. Kumar et al. (2005) reported significant decreased levels in AST, ALT, ALP and total bilirubin by methanolic extract of C. arborea. Ahmad et al. (2002) reported restoration of serum AST and ALT by Jigrin (a polyherbal formulation comprising C. arborea as one of the constituents). The present study is in agreement with these observations.

Kumar et al. (2008) observed a protective effect of methanol extract of C. arborea in N-Nitroso-diethylamine (NDEA) induced hepatocarcinogenesis by decreasing the activity of serum enzymes and bilirubin. Senthil Kumar et al. (2008) found antioxidant and hepatoprotective effect of methanol extract of C. arborea bark in ehrlich ascites carcinoma (EAC) by significant reversal of biochemical changes towards the normal in serum. Sairam et al.
(2016) reported hepatoprotective effect of Artocarpus altillis (Parkinson) Fosb. leaf and bark extracts against CCl₄ induced liver damage was assessed by analyzing improved activity of hepatic enzymes. Ahmad et al. (2012) revealed hepatoprotective effect of Semecarpus anacardium Linn. seed extract against ethanol induced hepatotoxicity by studying significant improved values of serum bilirubin, serum glutamate oxaloacetate (SGOT), serum glutamate pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP) and restoration of the normal histomorphological pattern of hepatocytes. The observations in the present study were in agreement with the literature cited. Measurement of the activities of marker enzymes, like AST and ALT can be used in the assessment of liver function (Ulican et al., 2008). Aspartate and aminotransferase are normally localized within the cells of liver, heart and kidney. The enzymes are of major importance in assessing and monitoring liver damage. Their presence in serum may give information on liver dysfunction. The liver cell injury induced by CCl₄ involves initially the metabolism of CCl₄ to trichloromethyl free radicals by the mix-function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link CCl₄ metabolism to the widespread disturbance in hepatocyte functions (Halliwell et al., 1992).

3.5 Histomorphological studies
Liver sections showing histomorphological architecture of all five groups of Wistar rats were presented in Figures A to E. In the group 1, the section of the liver was found to be normal, hepatocytes revealed normal anatomical pattern and healthy hepatocytes in vehicle control group (Figure A). The group 2 (Toxic group), section of the liver revealed severe fatty degenerative changes at peribulbal hepatocytes and centrilobular hepatocytes were comparatively normal (Figure B). The group 3 revealed peribulbal fatty degenerative changes, indicating the recovery of hepatocytes in the centrilobular spaces (Figure C). Section of the liver from group 4 showed mild granular and vacular degenerative changes in hepatocytes, leading to reduction of sinusoidal spaces. The nucleus of hepatocyte was intact, did not reveal necrotic area in the section (Figure D). The leucocytic infiltration in the section, indicating regenerative phase of liver. Group 5 revealed good regenerative effect on hepatocytes lumen. Centrilobular area revealed repaired hemorrhages and vacular degenerative mid lobular portion, however, peribulbal area revealed vacular degenerative changes, indicated active reparative phase in the group (Figure E). Ahmad et al. (2002) reported significantly reversal of thioacetamide induced hepatic toxicity by Jigrin (polyherbal preparation constituents C. arborea Roxb.) post treatment for 21 days, observed by normal central vein, hepatic cells with well preserved cytoplasm along with prominent nucleus and nucleolus. Natesan et al. (2007) reported anticancer potential of methanol extract of C. arborea against dalton’s lymphoma ascites (DLA)-induced ascites and liver solid tumors at different doses exhibited almost normal histological appearance of liver cell, except few lymphocytic collections in the portal area, indicating its potent hepatoprotective action. In present finding, repair of histological architecture of liver by extracts are in consonance with previously reported data. The improved histology of liver as seen in histopathological observations on animal treated with the plant bark extracts as compared to that seen in animals administered only CCl₄ indicated the possibility of plant material being able to induce accelerated regeneration of liver.

3.6 Gross morphological studies
Group T2 was received CCl₄, reveal congestion with necrotic foci and studded with nodular area. Liver showed decrease in organ weight and fragility, indicating toxic effect of CCl₄ gross pathological changes, in liver in the treatment groups did not reveal any prominent changes indicating the counter acting effect of CCl₄ by extract.

4. Conclusion
The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effects or restoring the normal hepatic physiology that has been attributed by hepatotoxin. Both aqueous extracts at different doses were decreased CCl₄ elevated liver enzyme level and improved histology of liver as seen in histopathological observations on animal treated with the plant bark aqueous extracts as compared to that seen in animals administered only CCl₄ indicated ameliorative effect of plant on hepatotoxicity. This suggests that the restoration of liver may be due to the nutraceutical nature of the stem bark of C. arborea.

Conflict of interest
We declare that we have no conflict of interest.

References