Hepatoprotective activity of *Trianthema decandra* on carbon tetrachloride-induced hepatotoxicity in rats

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The present study appraised the hepatoprotective activity of aqueous extract of *Trianthema decandra* roots against carbon tetrachloride-induced liver damage. Liver damage was induced by intraperitoneal administration of an equal mixture of carbon tetrachloride and olive oil (50% v/v, 0.5 ml/kg) in male Wistar rats (150-220 g) once daily for 7 days and the extent of damage was studied by assessing the biochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein and albumin in serum. The aqueous extract of *Trianthema decandra* roots (50 mg, 100 mg and 200 mg/kg) were orally administered to the animals with hepatotoxicity induced by carbon tetrachloride and its effects on biochemical parameters were compared with Silymarin (25 mg/kg) treated animals. *Trianthema decandra* (100 and 200 mg/kg) results in a significant reduction in serum hepatic enzymes when compared to rats treated with carbon tetrachloride alone. There was a significant increase in the serum total protein and albumin when compared to rats treated with carbon tetrachloride alone. The results concludes that the aqueous extract of *Trianthema decandra* roots (100 and 200 mg/kg) has protected the liver from carbon tetrachloride-induced damage.

Key words: Carbon tetrachloride, hepatoprotective and silymarin, *Trianthema decandra*

INTRODUCTION

During the past several decades, there has been a global trend for the revival of interest in the traditional system of medicine. Simultaneously, the need for the basic scientific investigation of medicinal plants using indigenous medical systems have became more interesting and relevant. *Trianthema decandra* is a prostrate weed belonging to the family Aizoaceae. It is distributed in the tropical and subtropical regions of the world. It is called Gdabani in Hindi and Vellai Shaaranai in Tamil. The root is used for the treatment hepatitis, asthma and orchitis, and also, the decoction of the root bark is credited with properties of aperients.[1] The juice of the leaves is dropped into the nostrils to relieve partial headache.[2] Its species *Trianthema portulacastrum* has significant hepatoprotective activity and also used in the treatment of oedema in liver and spleen.[3,4] The antioxidant activity of *Trianthema portulacastrum* has been reported earlier.[5] On the basis of the above reports, the present study has been undertaken to investigate the hepatoprotective activity of the aqueous extract of *Trianthema decandra* roots (AET) against carbon tetrachloride-induced liver damage in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Carbon tetrachloride (CCl_4) was obtained from E. Merck (India) Ltd., Mumbai. Silymarin was purchased from Micro labs, India. All other chemicals used in the study were of analytical grades.

Plant Material

The roots of *Trianthema decandra* were collected from mature plants during the month of October from the outskirts of Erode city. The plant samples were identified and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore, India. The voucher specimen (A 2459) has been deposited in Herbarium for ready references.

Extract Preparation

The collected roots of *Trianthema decandra* was washed, air-dried, powdered and extracted with distilled water using a Soxhlet extractor at room temperature. After exhaustive extraction, the collected aqueous extract was dried under reduced pressure using a rotatory flask evaporator and it was kept under refrigeration. This aqueous extract was used in further experiments.

Animals

Male Swiss albino mice weighing between 20 and 25 gm and

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male Wistar rats weighing between 150 and 220 gm were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30-70%. A 12:12 light:day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd., Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Acute Toxicity Studies

Acute toxicity studies were performed according to OECD-423 guidelines.[6] Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. AET was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of AET were employed for further toxicity studies.

Experimental Protocols

The animals were divided in to 5 groups of 6 animals each. Group I, which served as normal control received distilled water (1 ml/kg, p.o); Group II received equal mixture of CCl_4 and olive oil (50% v/v, 0.5 ml/kg i.p.) once daily for 7 days.[7] Group III received an equal mixture of CCl_4 and olive oil and AET (50 mg/kg, p.o) simultaneously once daily for 7 days. Group IV received equal mixture of CCl_4 and olive oil and AET (100 mg/kg, p.o) simultaneously once daily for 7 days. Group V received equal mixture of CCl_4 and olive oil and AET (200 mg/kg, p.o) simultaneously once daily for 7 days. Group VI received equal mixture of CCl_4 and olive oil and Silymarin (25 mg/kg, p.o) simultaneously once daily for 7 days.[8] On the eighth day, the blood was collected by direct cardiac puncture under light ether anaesthesia and serum was separated for various biochemical estimations. The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed in serum using standard kits from Lupin Laboratories and Pointe Scientifics. The results were expressed as units/L (U/L). The levels of proteins, i.e., total proteins and albumins were estimated in serum of experimental animals by earlier method reported.[9]

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't'-test. P values <0.05 were considered to be significant.

RESULTS

Acute Toxicity Studies

All the doses (5, 50, 300 and 2000 mg/kg) of AET employed for acute oral toxicity studies were found to be non-toxic. AET did not produce any mortality even at the highest dose (2000 mg/kg) employed. Three submaximal doses (50, 100 and 200 mg/kg), which were found to be safe, were employed for further pharmacological investigations.

Biochemical Estimations

The results of hepatoprotective activity of AET on CCl_4-treated rats are shown in [Figs. 1 and 2]. The hepatic enzymes ALT, AST, and ALP in serum significantly ($P < 0.001$) increased in CCl_4 treated animals when compared to control. The AET treatment (50 mg/kg) did not have significant effect on the levels of hepatic enzymes when compared to CCl_4-treated animals. The AET treatments (100 and 200 mg/kg) significantly ($P < 0.05$, $P < 0.01$; respectively) reversed the levels of hepatic enzymes when compared to CCl_4-treated animals. Silymarin (25 mg/kg)-treated animals also showed significant ($P < 0.01$) inverted the levels of hepatic enzymes when compared to CCl_4-treated animals. There was a significant decrease ($P < 0.001$) in the serum total protein and albumin levels in CCl_4 treated groups when as compared to the control group, which was significantly ($P < 0.01$ and $P < 0.05$) reversed with the treatment of AET (200 mg/kg) and AET (100 mg/kg), respectively.

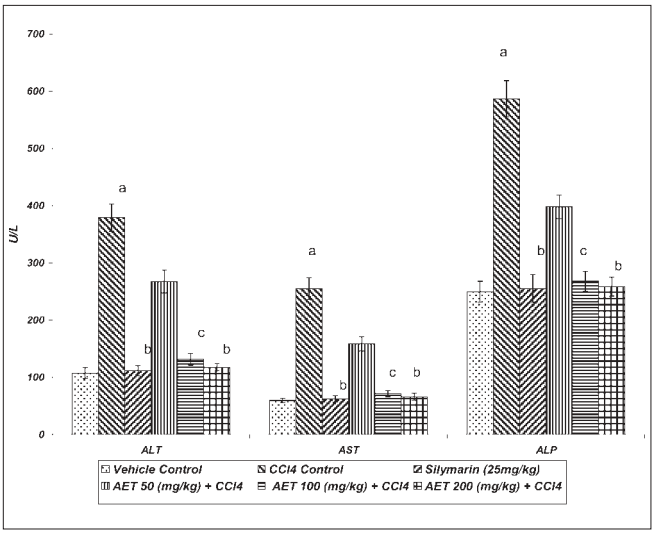

Figure 1: Effect of aqueous extract of *Trianthema decandra* roots on serum ALT, AST and ALP in CCl_4-induced hepatotoxicity rats after 7 days treatment. Values are in Mean ± SEM ($n = 6$): [a]$P < 0.001$ Vs Group I; [b]$P < 0.01$ Vs Group II; [c]$P < 0.05$ Vs Group II

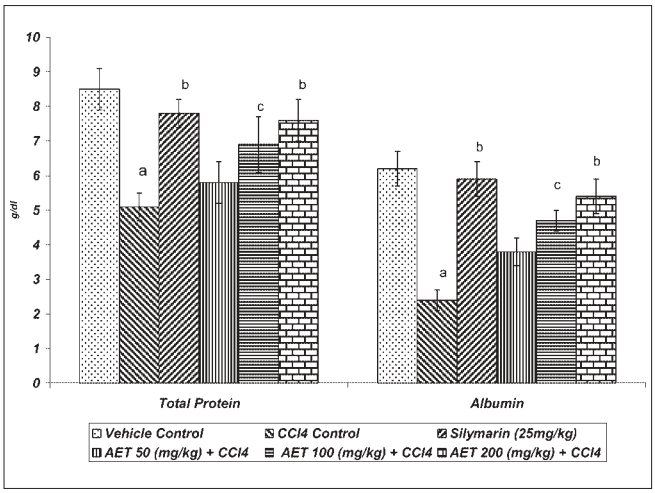

Figure 2: Effect of aqueous extract of *Trianthema decandra* roots on serum total protein and albumin in CCl_4-induced hepatotoxicity rats after 7 days of treatment. Values are in Mean ± SEM ($n = 6$): $^aP < 0.001$ Vs Group I; $^bP < 0.01$ Vs Group II; $^cP < 0.05$ Vs Group II

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease.[10] The assessment of liver function can be made by estimating the activities of serum enzymes such as ALT, AST and ALP. During hepatic damage, there may be increase in these enzyme levels in serum with the extent of liver damage. The altered levels of these enzymes in CCl_4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin.

The present study has demonstrated that AET (100 and 200 mg/kg) exhibited significant dose-dependent hepatoprotective activity against liver injury induced by CCl_4. Carbon tetrachloride induces hepato-toxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Carbon tetrachloride is metabolically activated by the cytochrome P450 in the endoplasmic reticulum to form a trichloromethyl free radical ($ccl_3^{\bullet}$) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, which leads to change in the structures of endoplasmic reticulum and other membrane, loss of metabolic enzymes activation, reduction of protein synthesis and elevation of serum transaminases leading to liver damage.[11] Amino transferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-ketocids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect of various compounds.[12] Both AST and ALT levels increase due to toxic compounds affecting the integrity of liver cells.[13] Alkaline phosphatase is a membrane bound glycoprotein enzyme with a high concentration in sinusoids and endothelium. This enzyme reaches the liver mainly from the bone. It is excreted into the bile; therefore, its elevation in serum occurs in hepatobiliary diseases.[14] The results of the present study indicate that AET probably stabilize the hepatic plasma membrane from CCl_4-induced damage.

The liver is known to play a significant role in the serum protein synthesis, being the source of plasma albumin and fibrinogen and also the other important components like α and β- globulin. The liver is also concerned with the synthesis of γ-globulin. The serum albumin level is low in hepatic diseases. The result reveals that in the animals pre-treated with hepatoprotective agents prior to the challenge with CCl_4, the liver biosynthesis of protein continues to be unaffected. The metabolic transformation of amino acid in liver by synthesis, transamination, etc., may be impaired due to the escape of both non-proteins and protein nitrogenous substances from injured liver cells as mediated by raise in the serum enzyme levels of ALP, AST and ALT. The protective activity of the extracts may be attributed to the membrane stabilizing agents present in the AET, which may avert enzyme leakages in tissues in response to CCl_4 poisoning leading to enhanced metabolic transformation of amino acids in liver through synthesis and transformation.[15] AET enhanced the synthesis of TP and Albumin which accelerates the regeneration process and the protection of liver cells. Therefore, the increased level of total protein in serum indicates the hepatoprotective activity of AET. The effects of AET (200 mg/kg) were comparable with the effects of Silymarin-treated groups.

It can be concluded that of aqueous extract of *Trianthema decandra* root possess hepatoprotective activity against CCl_4-induced liver damage in rats. According to the results obtained in this study, it may be inferred that, in general, AET reverses the hepatic damage induced by CCl_4. To the best of our knowledge, this is the first report about *in vivo* activity of *Trianthema decandra* and seems to raise some concern about the traditional indications of this species as a medicine for liver diseases. Certainly, further studies need to be carried out with other hepatotoxic compounds to prove the hepatoprotective efficacy of *Trianthema decandra.*

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