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***In vivo* hepatoprotective effect of *Trianthema decandra* extracts on carbon tetrachloride induced rats**

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ABSTRACT

In the present investigation, an attempt has been made to test the hepatoprotective efficacy of the herbal plant, Trianthema decandra on carbon tetrachloride induced toxic hepatitis. The carbon tetrachloride induced rats showed higher level of total protein (TP), total cholesterol (TC), triglycerides (TG), Alkaline Phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) and bilirubin in serum. The leaf extract of T. decandra was treated with hepatitis rats showed remarkable reduction in the activity of TP, TC, TG, ALP, AST, ALT and bilirubin when compared to the normal rats. This research suggests that the crude extract of T. decandra could control the carbon tetrachloride induced hepatitis.

Keywords: Medicinal plant, anti-hepatitis, enzymes, bilirubin.

INTRODUCTION

Liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. The liver is normally involved in the metabolism of proteins, fats and carbohydrates. Hepatitis or inflammatory disorder involves inflammation and damage to the hepatocytes. Every year 18,000 people had been reported to die due to liver cirrhosis caused by viral hepatitis [1]. Liver being the organ of biotransformations of various xenobiotics such as alcohol, drugs, industrial and agricultural chemicals often takes the brunt of severe damage. Alcohol consumption is a major

threat of liver disorder; alcohol induced liver injury progresses from fatty infiltration and following pernicious course of inflammation leading to irreversible damage. It is well known that chronic ethanol ingestion produces fatty liver, hepatomegaly, alcoholic hepatitis, fibrosis and cirrhosis [2]. Interaction between alcohol and other hepatotoxic agents such as chemicals, drugs [3] and chronic hepatitis B and c infections excess hepatic iron and endotoxins are also important in acute and chronic liver injury. In Indian system of medicines particularly in Ayurveda and siddha, large number of plants based formulations are advocated to cure various ailments. A large group of native plants have been screened and proved to be effective in curing liver disorders. The search and screening of more and more plants with more effective potential is still continuing, because the hidden potential of many plants has not been fully explored and revealed. Keeping this in mind, the present study is undertaken up to assess the efficacy of a commonly available plant species, *Trianthema decandra* in curing carbon tetrachloride induced toxicity, with special emphasize on relevant biochemical mode of action

EXPERIMENTAL SECTION

Plant material

The plant *Trianthema decandra* was selected for this study. This plant belonging to the family, Aizoaceae is commonly found all over rural Tamilnadu. One of related species *Trianthema portulacastrum* has been reported to have hepatoprotective effect. Though *T. decandra* is widely used in siddha medicine for various disorders of eye, stomach indigestion, inflammation, convulsions but the hepatoprotective effect has not yet evaluated.

Experimental animal and Chemicals

The healthy adult albino rats were used as experimental animals for this research. Adult rats of both sexes of 10 weeks old weighing about 150-200g were selected and segregated in polypropylene cages. Fresh dry husk was used as bed material. They were acclimated to the laboratory condition for a week period prior to the start of experiments. They were fed with rat feed purchased from Hindustan lever Ltd., Bangalore, and water ad libitum and were used for the study after prior securitization and approval from Institutional Animal Ethical committee (IAEC). Carbon tetrachloride (CCl₄) was used for the induction of hepatic toxic and it was purchased from Sigma-Aldrich chemicals pvt Ltd, Bangalore, India and other routine chemicals and reagents used were of analytical grade.

Drug preparation

Fresh healthy plants free from pest infection were collected from wild. The aerial parts were separated and washed in running water several times to get rid off soil, microbes, eggs of insect pest, etc. samples were cut in to small pieces and air-dried for 24 hours at room temperature before extraction with solvent. Again the sample pieces were rinsed with sterile distilled water and the tissue samples were used for extraction using alcohol. The extract was cold steeped overnight at -18⁰ C and filtered with Whatman No.1 filter paper. The filtrate was poured in previously weighed Petri plate and evaporated to dryness in rotary evaporator [4], [5], [6]. The dried crude extracts were used for hepatoprotective test in carbon tetrachloride induced animals. The extracts were transferred to small (50ml) beakers and vacuum dried in a dessicator. The slurry powdered alcohol extract were weighed and used to prepare the required dose by mixing in suitable volume of groundnut oil (1.5g in 10ml).

Induction of liver disease

The animals were divided into 3 groups with 6 rats in each group of rats was maintained with only food and water and treated as control and the remaining groups were administered intraperitoneally with ccl4 at the dose level of 0.5ml/kg bodyweight daily for 15 days. One of the ccl4 treated groups was maintained with recovery and the remaining groups of rats were treated with test plant extract mixed with the carrier oil. At the end of 15days, the rats were anesthetized and the blood was collected from ocular vein. Serum was separated and stored in anticoagulated tubes for future biochemical tests. Marker enzymes such as aspartate transaminase (AST), Alaninetransaminase (ALT) were estimated and Alkaline phosphatase (ALP) was carried out by following the method of Reitman and Frankel [7], bilirubin was estimated by the method of Melloy and Evelyn [8] and Total serum protein (TSP), Total cholesterol, triglycerides were also estimated. Student's t test was used for statistical significance between groups.

Serum Biochemical parameters

Table:1 shows the status of total protein, triglycerides, Total cholesterol and bilirubin levels estimated in serum of the control and carbon tetrachloride treated rats. The total protein content decreased in ccl4 induced control rats when compared to control rats. The Total protein content was significantly restored in ccl4 induced rats treated with *Trianthema decandra* herbal extract. Table 2 shows the results of ALP, AST, ALT contents increased in ccl4 treated rats and were well maintained in herbal extract showed much restoration activity.

RESULTS AND DISCUSSION

The total protein content decreased in ccl4 induced control rats.(5.32±0.673) when compared to control rats (6.545±0.915).The Total protein content was significantly restored in ccl4 induced rats treated with *Trianthema decandra* herbal extract (6.157±1.0115). Triglycerides levels shows decreased in ccl4 induced rats (106.45±2.231) when compared to control rats (129.5±1.272) and triglycerides level in alcohol extract treated group is more nearer to the control values (129.747±1.097). The total cholesterol is found to be reduced significantly in ccl4 treated rats (63.36±1.085). when compared to control (74.45±0.88) when compared to the respective ccl4 treated normal group, I is evident that in the plant extract treated group the cholesterol level is significantly higher and more nearer to the control values. The Bilirubin content increased in ccl4 treated rats (0.63±0.08) and were well maintained in herbal extract (0.68±0.05).Table (2) shown the results of Alkaline Phosphatase, Aspartate transaminase, Alanine transaminase contents increase in ccl4 treated rats (100.5±7.5, 160.0±11.0 and 70.2±6.51 IU/L respectively) and were maintained in herbal extract showed much restoration activity (55.5±1.91,121.5±9.71 and 85.2±6.51 IU/L respectively).

Table:1. Shows the Biochemical parameters of normal and hepatotoxic rats

Serum	Total proteins (mg)	Total cholesterol	Triglycerides	Bilirubin (mg/dl)
Control	6.545±0.95	74.45±0.88	129.5±1.272	0.55±0.12
Control+ccl4	5.32±0.673 *	63.36±1.085 *	106.45±2.231 *	0.53±0.08 *
Ccl4+alcohol extract	6.157±1.01 **	76.225±1.61 **	129.747±1.09 **	0.68±0.05 **

Values are compared as mean ±S.E

P<0.01=P<0.05=Significant

*P<0.01 when compared with group I

**P<0.05 when compared with group II

Table 2 Shows the enzymes Biochemical parameters of normal and Hepatotoxic rats

Serum	Alkaline phosphatase (U/L)	Aspartate transaminase (U/L)	Alanine transaminase (U/L)
Control	48.18±12.0	50.14±4.02	40.12±7.22
Control+CCl ₄	100.5±7.5 *	160±11.0 *	70.2±6.51 *
CCl ₄ +alcohol extract	55.25±1.91 * *	121.5±9.71 * *	85.2±6.51 * *

Values are compared as mean ±S.E

P<0.01=P<0.05=Significant

*P<0.01 when compared with group I

* *P<0.05 when compared with group II

Levels of all marker enzymes increased significantly in group II rats after ccl4 administration (p<0.0001), as compared to normal controls (Table 1). *Trianthema decandra* treatment caused significant decrease in the activities of all these enzymes through the decrease. The increased activities of the liver marker enzymes such as ALT, AST, ALP in serum of ccl4 induced rats indicate damage to hepatic cells [9]. Damage to the cell integrity of the liver by ccl4 is reflected by an increase in the activity of AST, which is released into circulation after cellular damage. ALP is an coenzyme of the hepatocyte plasma membrane. Carbon tetrachloride mediated acute toxicity increased permeability of the hepatocyte membrane and cellular leakage [10]. The present study concurs with the above reports. The *Trianthema decandra* mediated suppression of the increased AST, ALT, ALP and bilirubin activities suggested the possibility of the extract to give protection against liver injury upon ccl4 induction. The decrease in TSP observed in ccl4 treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into the decreased hepatic capacity to synthesize protein. But restoration of the level of TSP after the administration of *T. decandra* confirmed the hepatoprotective nature of *T. decandra*.

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