PROTECTIVE EFFECT OF AQUEOUS LEAF EXTRACT OF ALTERNANTHERA SESSILIS L. ON CCL4 INDUCED HEPATIC DAMAGE IN ALBINO MICE

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Summary

Alternanthera sessilis L. was used extensively in the indigenous system of medicine as a hepatoprotective agent. The current investigation focuses on the hepatoprotective activity of Alternanthera sessilis L. on CCl₄ induced hepatic damage in albino mice. One group of animals were induced CCl₄ (0.5ml/kg body wt- in olive oil-ip) single dose. The serum liver marker enzyme like ALT, AST, ALP and Bilirubin were estimated in CCl₄ induced animals. The toxicity induced animals the levels of marker enzymes were significantly increased. The CCl₄ induced animals were fed with plant extract 250mg/kg body wt., orally by intragastric tubes for 7 days. The levels of serum marker enzymes of plant extract fed animals were significantly reduced (P<0.001), when compared to normal and control animals.

Key words: *Alternanthera sessilis* L., CCl₄ (Carbon tetrachloride), Alanine transaminase (ALT), Asparate transaminase (AST), Alkaline phospatase (ALP).

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Introduction

Liver is an important organ actively involved in metabolic functions, production and secretion of bile, production of prothrombin and fibrinogen, processing of dietary amino acids carbohydrates, lipids, vitamins and synthesis of serum protein. The liver is also responsible for detoxifying poisonous substances in the body by transforming and removing toxins, waste and pollutant xenobiotics (1).

Liver injury due to chemicals or infectious agents may lead to progressive liver fibrosis and, ultimately, cirrhosis and liver failure (2). However, no effective treatment that delays disease progression and complications has yet been found. Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material (3). The scientific studies available on a good number of medicinal plants indicate that promising phytochemical can be developed for many health problems (4). Several recent studies suggest that traditional herbs and micronutrients such as carotenoids and selenium may be useful for this purpose (5,6).

Carbon tetrachloride CCl₄ is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals (7).CCl₄ is a selective hepato-toxic chemical agent. CCl₄ induced reactive free radicals initiative cell damage through two different mechanisms of covalent blinding to the membrane proteins and cause lipid per oxidation. A number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals (8). Production of reactive oxygen species and lipid per oxidation induced by iron overload (9) cholestatic injury(10) and intoxication by ethanol (11) and CCl₄ is associated with liver fibrosis and cirrhosis. Hepatic damage induced by CCl₄ resulting in an increase in serum aspartate transaminase (AST) and serum alanine transaminase (ALT) concentration (12). The elevation of concentrations of serum enzymes such as AST and ALT is generally regarding as one of the sensitive markers of hepatic damage (13). The present study was undertaken, to evaluate the use of Alternanthera sessilis leaf extracts as a hepatoprotective agent on carbon tetrachloride induced hepatotoxicity in *albino* mice.

Materials and methods

Plant material

The Alternanthera sessilis L. is the folklore medicinal plant were collected in and around vellore, Tamilnadu, India. The leaves were selected for the hepatoprotective studies. The leaves were cleaned and shade dried at room temperature and authenticated (No.CAHC/01/2009) by the department of Botany, C. Abdul Hakeem College, Tamilnadu. The shade dried plant material was powdered by using electric blender.

Plant extracts preparation

100gms of powdered plant leaf materials were taken and mixed with 500ml of distilled water and were magnetically stirred in separate containers of overnight at room temperature. The residue was removed by filtration and the aqueous extracts were

concentrated under vacuum to get solid yield of 10% leaf. The plant extracts were administered to animals in aqueous solution.

Animals

Adult male *albino* mice of Wistar strain weighing around 25 to 30gms were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were kept in polypropylene cages (four in each cage) at an ambient temperature of $25\pm2^{\circ}$ C and 55-65% relative humidity. A 12 ± 1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional animal ethics committee.

Estimation of biochemical parameters

The blood samples were collected from direct cardiac puncture with light anesthesia. The serum was separated for estimation of biochemical parameters. The marker enzymes (AST, ALT and ALP) were studied by Bergmeyer method (14) and kind, Amstrong method (15) respectively.

Experimental design

Group 1: Normal animals.Group 2: Control animals – Olive oil treated (0.5 ml/kg body wt).Group 3: CCl₄ injected animals (0.5 ml/kg body wt. in olive oil-ip)for single dose.Group4:CCl₄ injected animals were administrated with plant extract for 7 days.

Results

The results showed in the table - 1 are exhibited the significant hepatoprotective effects of the plant *Alternanthera sessilis* L. The activity of levels of serum Asparate transaminase (AST), Alanine transaminase (ALT), Alkaline Phosphate (ALP), and bilirubin were taken as an index for hepatotoxicity induced by CCl₄. The levels of AST, ALT, ALP and the levels of bilirubin were analyzed in serum samples of different groups of mice shown in table 1. Serum marker enzymes such as ALT, AST, ALP and bilirubin were analyzed to control and experimental animals. The group II (control) olive oil treated animals, the level of marker enzymes were not significantly elevated when compared to the normal group I animals. The level of marker enzymes of group III CCl₄ induced animals were significantly increased (P<0.05) when compared to the normal and control animals. But when the aqueous extract of *Alternanthera sessilis* leaf extract was given in group IV, there was a significant (P<0.001) decrease in the value, which tends to reach the normal and control values. Carbon tetracholoride is reported to produce free radicals, which affect the cellular permeability of hepotocytes leading to elevated levels of serum biochemical parameters like ALT, AST, ALP and bilirubin.

Discussion

The present study was performed to assess the hepatoprotective activity in mice against carbon tetrachloride (CCl_4) as hepatotoxin to prove its claims in folklore medicinal plant practice against liver disorders. The observed protective effect of the plant extract against carbontetrachloride may be attributed to the presence of flavonoids,

ascorbic acid, carotenoids, tannis and lignins among the plant constituents (16). Flavonoids are known to be antioxidants, free radical scavengers and anti-lipoperoxidants leading hepatoprotection. Many compound known to be beneficial against CCl₄ mediated liver injury exert their protective action by toxin-mediated lipid per oxidation either via a decreased production of CCl₄ derived free radicals or through the antioxidant activity of the protective agents themselves (17). The depleted levels of AST, ALT, ALP and bilirubin in serum might be due to the decreased leakage of the enzymes form the cytosol of the liver tissue indicating a restoration of liver cell membrane disturbance byCCl₄. From the table 1 it was evident that the plant extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication.

Table-1: Effect of Alternanthera sessilis L. (Aqueous leaf extract) on liver marker
enzymes in the serum of control and experimental animals

Name of the test	Normal	Olive oil- treated Control	CCl4- treated mice	Plant extract treated mice
Bilirubin- Total (mg/%)	0.4±0.02	0.4±0.02 NS	0.6±0.04**	0.4±0.02**
AST(U/L)	120±1.67	123±1.87 NS	165±3.42**	123±2.62**
ALT(U/L)	32±2.52	35±2.24 NS	57±2.42**	34±2.4**
ALP(U/L)	210±2.13	214±2.16 NS	290±3.42**	215±2.61**

Keys: NS- Non significant, P denotes statistical significant * P<0.05, ** P<0.001.

The results obtained in the present study regarding the effect of CCl_4 intoxication on the concentrations of ALT and AST are in agreement with those reported (12). This report showed that CCl_4 intoxication induces changes in the process of protein synthesis and the levels of DNA are decreased and this is accompanied with increased concentrations of ALT and AST. However, the reduced concentration of ALT and AST as a result of plant extract administration observed during the present study might probably be due in part to the presence of catechins in the extract (18).

To conclude that hepatoprotective effect of *Alternanthera sessilis* might play a major role in controlling the tissue damage caused by reactive oxygen species. The biochemical studies confirmed the protective effect of the aqueous extract of *Alternanthera sessilis* against CCl₄ induced liver damage in mice.

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