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Hepatoprotective potential of petroleum ether leaf extract of *Crossandra infundibuliformis* on CCl_4 induced liver toxicity in albino mice

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ABSTRACT

Objective: To analyze the hepatoprotective effect of the *Crossandra infundibuliformis*. **Methods:** Hepatotoxicity was induced by carbon tetrachloride. Petroleum ether extract of dried leaves was administrated to mice for 7 days. The hepatoprotective effect of petroleum ether extract was evaluated by the assay of liver function biochemical parameters. **Results:** The result clearly indicates that petroleum ether extract showed significant hepatoprotection when compared with standard Silumarin. **Conclusions:** The petroleum ether extract of the leaves of *Crossandra infundibuliformis* possess significant acute hepatoprotective activity. Thus further investigation on this species would bring a promising drug for liver disorders.

1. Introduction

The medicinal plants were a common link between traditional and modern medical sciences as they were the main source of drugs and medicaments[1]. Liver is the largest organ in the vertebrate body and the site for intense metabolism. The liver is also responsible for detoxifying poisonous substances in the body by transforming and removing toxins, waste and pollutant xenobiotics[2,3]. Liver diseases remain one of the serious health problems and caused by various agents like alcohol consumption, environmental toxins and viruses, which remains as one of the major threats to public health. The Indian traditional system of medicine, especially herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost. However, we are not aware of a satisfactory remedy for serious liver diseases and search for effective and safe drugs for liver disorders continues to be an area of interest.

Carbon tetrachloride, CCl_4 is one of the oldest and most widely used toxins for experimental induction of liver fibrosis in laboratory animals and it is a selective hepato-toxic chemical agent. CCl_4 induced reactive free radicals initiate cell damage through two different mechanisms of covalent blinding to the membrane proteins and cause lipid peroxidation. The principal cause of carbon tetrachloride induced hepatic damage is lipid peroxidation and decreased activities of antioxidant enzymes and generation of free radicals. The resulting hepatic injury was characterized by leakage of cellular enzymes into the blood stream and by centrilobular necrosis. A number of investigators have utilized this chemical to produce liver cirrhosis in experimental animals[4]. Production of reactive oxygen species and lipid peroxidation induced by iron overload[5], cholestatic injury[6] and in toxication by ethanol[7] and CCl_4 is associated with liver fibrosis and cirrhosis. Hepatic damage induced by CCl_4 resulting in an increase in serum aspartate transaminase (AST) and serum alanine transaminase (ALT) concentration[8]. The elevation of concentrations of serum enzymes such as AST and ALT is generally regarded as one of the sensitive markers of hepatic damage[9]. CCl_4 is known to have a profound effect on the metabolism of lipids and lipoproteins. Accumulation of lipids in the hepatocytes is the most striking initial manifestation of CCl_4 induced liver injury.

The importance of herbal medicines in curing various ailments has already been established. There are potent indigenous herbal medicines available for liver disorders in various parts of the world and most of them have not yet been scientifically validated and if done may lead to the

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development of cost effective drugs. Hence our research were targetted towards the potent herbal medicine to cure liver disorders. In continuation of our previous work on antimicrobial and free radical scavenging activity of crude extracts[10,11] clearly suggests that petroleum ether extract has biologically active compounds compared to ethylacetate and methanol extract. Henceforth we are reporting the hepatoprotective activity of the petroleum ether extract.

2. Materials and methods

All the materials used in this study were of analytical grade purchased from S.D. Fine Chemicals, Mumbai, India. The biochemical estimations were carried out using kit purchased from Span Diagnostic, Surat, India. Solvents and reagents were commercially sourced and used without further purification.

2.1. Plant collection and preparation

The leaves of *Crossandra infundibuliformis* (Family: Acanthaceae) (*C. infundibuliformis)* were collected in and around Vellore District, Tamil Nadu. The plant material was authenticated (No: BSI/SC/5/23/09-10/Tech.-1718) by the Botanical Survey of India, Coimbatore, Tamil Nadu. The collected leaves were cleaned with distilled water, shade dried at room temperature. The air dried plant materials were powdered separately in an electrical blender and stored at 5 ℃ until further use. 100 gms of the dried leaf powder were taken separately and it was extracted by petroleum ether by using Soxhlet apparatus. The residue was removed by filtration and the petroleum ether extract were concentrated under vacuum to get solid greenish yellow yield.

2.2. Animals

Adult male Swiss albino mice weighing around 25-30 g were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each) at an ambient temperature of (25±2) ℃ and 55%-65% relative humidity (12 ±1) h 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). They had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines (No. 1011/c/06/CPCSEA).

2.3. Experimental design

Group 1: Normal animals.

Group 2: Control animals - Olive oil treated (0.5 ml/kg body wt).

Group 3: CCl_4 injected animals (0.5 ml/kg body wt. in olive oil-ip) for single dose.

Group 4: CCl_4 injected animals were administrated with plant extract for 7 days.

2.4. Estimation of biochemical parameters

The biochemical parameters like serum enzymes were analyzed. They included ALT and AST, alkaline phosphatase (ALP)[12], serum bilurubin[13], Total cholesterol, TG, free fatty acids and phospholipids[14].

2.5. Statistical analysis

All results were expressed as mean±SD (n=3). The percentages of mortality were determined and transformed to descriptive statistics using Sigma plot 11.0.

3. Results

The activity levels of ALT, AST, ALP and bilirubin were taken as an index for hepatotoxicity induced by CCl_4.

The levels of biochemical marker enzymes in olive oil treated Group 2 animals were insignificantly increased when compared to the levels in normal groups. The CCl_4 induced groups the levels of biochemical marker enzymes such as ALT, AST, ALP and Bilirubin were significantly (P<0.001) elevated by 75.23%, 41.25%, 52.66% and 29.40% respectably when compared to the levels in normal mice. After administration of plant extract the increased levels of marker enzymes were found to have decreased significantly by 36.59%, 26.54%, 30.13% and 73.33% respectively when compared to the levels in CCl_4 induced groups.

The lipid profile like total cholesterol, triglycerides, free fatty acids and phospholipids were observed in CCl_4 and plant treated groups. The levels of lipid profile were insignificantly increased in olive oil treated control groups. In group 3 animals were treated with CCl_4, the levels of lipid profile was significantly increased by 43.5%, 90.76%, 104.80% and 43.66% respectively when compared to the levels in normal groups. After administration of *C. infundibuliformis* (250 mg/kg body wt) the elevated levels were significantly deceased by 26.20%, 41.82%, 43.66% and 23.87% respectively when compared to the levels in CCl_4 induced control groups.

4. Discussion

The levels of the cellular marker enzymes *viz*., ALT, AST, ALP and bilirubin in serum reflect the physiological state of the liver. The levels of these enzymes change according to distortion of the liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases. The increased levels of AST, ALT, ALP and serum bilirubin are conventional indicators of liver injury. The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effects or in maintaining the normal hepatic physiological mechanisms which have been altered by a hepatotoxin.

In our experiments when CCl_4 was given to the experimental animals, the levels of cellular marker enzymes were found to have been significantly elevated in them indicating increased cellular permeability, damage and necrosis of hepatocytes which might be due to the leakage of the cellular enzymes into the serum. The significant decrease

Table 1
Effect of petroleum ether extract of *C. infundibuliformis* leaves on liver marker enzymes(1 μ /L/min/mg protein) and bilirubin (mg/dL).

Experimental groups	ALT	AST	ALP	Bilirubin
Normal	31.50± .52	119.50±1.67	210.00±2.13	0.38±0.02
Olive oil	33.50±2.24	123.00±1.87	217.18±2.18	0.39±0.02
CCl_4 induced	55.20±2.41*	168.80±3.42*	320.60±3.42*	1.50±0.08*
Plant fed	35.00±2.40*	124.00±2.62*	224.00±2.67*	0.40±0.02*

*P<0.01, % of changes comparing with normal treated group.

Table 2
Effect of petroleum ether extract on lipid profile(μ g/dL).

Experimental groups	Total cholesterol	Triglycerides	Free fatty acid	Phospholipids
Normal	101.00±2.28	54.60±1.75	52.00±1.89	79.70±1.76
Olive oil	105.00±2.20	56.70±1.61	54.00±1.78	81.00±1.47
CCl_4 induced	145.00±2.58*	104.16±2.56*	106.50±2.73*	114.50±3.56*
Plant fed	107.00±0.89*	60.60±1.63*	60.00±1.41*	87.16±1.60*

*P<0.01, % of changes comparing with normal treated group.

of cellular enzymes in *C. infundibuliformis* plant extract treated groups might be due to decreased leakage from the hepatic cells. This suggests that the *C. infundibuliformis* plant extract could repair the hepatic injury and or restore the cellular permeability, thus reducing the toxic effect of CCl_4 damaged liver tissue.

The CCl_4 acts as a surfactant and suppresses the action of enzyme LCAT to block the uptake of lipoprotein from circulation by extra hepatic tissue, resulting in an increase in blood lipid levels. The hyper-lipedemic condition revealed in the serum of CCl_4 administered animals was restored to normal after the supplementation of the plant extract. The decreased serum cholesterol in the plant extract administrated mice might be due to increased activity of enzyme LCAT involved in esterification of cholesterol in the plasma. The significant decrease in the triglycerides (TG) in serum in the *C. infundibuliformis* plant extract administrated animals might be due to decreased accumulation of lipoprotein. This might be due to increased activity of lipoprotein lipase, which is involved in the uptake of TG rich lipoprotein by extra hepatic tissue. The significant decrease in the free fatty acid accumulation in serum of plant extract administrated animals might be due to decreased lipid breakdown, which corroborates with results obtained where in a decreased lipid peroxidation. The significant decrease of the phospholipids in the serum of plant extract administrated animals might be due to decreased peroxidation in the biomembrane of hepatocytes.

Conflict of interest statement

We declare that we have no conflict of interest.

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