Restorative effect of *Eclipta alba* in CCl₄ induced hepatotoxicity in male albino rats

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ABSTRACT

Objective: To elucidate the restorative effect of the aqueous leaf extract (85%) of the traditional medicinal plant *Eclipta alba* (*E. alba*) against CCl₄ induced hepatotoxicity in male albino rats.

Methods: Restorative activity was assessed using CCl₄–induced hepatic injury in rats by monitoring biochemical parameters. Biochemical markers of hepatic damage such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assessed in serum. The oxidative stress was evaluated by measuring the levels of thiobarbituric acid reactive substance (TBARS), hydroperoxides, activity levels of enzymes viz. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in hepatic tissue.

Results: CCl₄ and olive oil mixture (1:1 dosage of 1 mL/kg b.w.) induced oxidative stress was indicated by elevated levels of TBARS and hydroperoxides, and augmented levels of serum AST, ALT and ALP. The depleted activity levels of antioxidant enzymes such as SOD, CAT, GPX and GST were found in CCl₄ induced animals. The aqueous leaf extract of *E. alba* (250 mg/kg b.w.) ameliorated the effects of CCl₄ and returned the altered levels of the biochemical markers near to the normal levels.

Conclusions: The study indicates that aqueous leaf extract of *E. alba* has potential restorative effect on CCl₄ induced hepatotoxicity in male albino rats.

1. Introduction

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic function and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals[1]. The potentially reactive oxygen species, ascribed as ROS, such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (OH⁻), are continuously generated inside the human body as a consequence of the exposure to exogenous chemicals in our environment and/or to a number of endogenous metabolic processes involving redox enzymes and bioenergetic electron transfer[2]. Therefore, there has been considerable interest in role of complementary and alternative medicines for the treatment of liver disease. Developing therapeutically effective agents from natural products may reduce the risk of toxicity when the drug is used clinically[3]. Chemicals such as carbon tetrachloride (CCl₄) catalysed radicals induced lipid peroxidation, damaged the membranes of liver cells and organelles, caused the swelling and necrosis of hepatocytes and resulted in the release of cytosolic enzymes such as alanine transferase (ALT), aspartate transaminase (AST) and alkaline phosphates (ALP) into the blood circulation[4,5]. The major component of the antioxidant system in mammalian cells consists of three enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). These enzymes work in concert to detoxify superoxide anion and H₂O₂ in cells. Therefore, reducing oxidative stress may be an effective therapeutic strategy for preventing and treating hepatic fibrosis[6]. The initial step in the tissue injury induced by CCl₄ is its cytochrome P450–mediated transfer of a single electron to the C–Cl bond, giving a radical anion as a transient intermediate that eliminates chlorine to form a carbon centered radical, the trichloromethyl radical (CCl₃) and chloride[7]. *Eclipta alba* (*E. alba*) is an annual herb that is hardy and non-invasive. The plant grows to approximately two feet in height, with a thin woody stem, dark green leaves and small white flowers. The aerial parts...
of the plant, such as the leaves, flowers and upper stems, are used medicinally. It is commonly called as Vellai karisilankani in Tamil. *E. alba* is mainly used in hair oils, but it has been considered a good drug in hepatotoxicity. It may be used to prevent habitual abortion and miscarriage and also in cases of post-delivery, uterine part. A decoction of leaves is used in uterine hemorrhage. The juice of the plant with honey given to infants with castor, act on the expulsion of worms. Leaf is used for antihyperglycemic[8], antibacterial[9], anti-inflammatory activities[10] and may be applied to insect bites, stings, swellings, and other skin diseases[11]. In traditional Chinese medicine, *E. alba* is considered a nourishing herb for the liver and kidney. It is often used in combination with *Ligustrum* and *Lyceum* to boost the immune system[12]. The present study is under taken to elucidate the restorative effect of the aqueous leaf extract of the folklore medicinal plant *E. alba* against CCl₄ induced hepatic toxicity in male albino rats.

2. Materials and methods

2.1. Plant material

*E. alba* was identified, and the leaves were collected during the month of September–January (2010–2011) in and around Vellore District, Tamilnadu, India. The leaves were cleaned, shade dried, authenticated and voucher specimen was deposited in the Department of Botany, Voorhees College, Vellore, Tamilnadu.

2.2. Preparation of plant extract

The shade dried leaves were powdered separately in an electric blender and stored at 5 °C until further use. The leaf powder (100 g) was taken and mixed with 500 mL of distilled water and stirred magnetically in separate containers overnight at room temperature. The residue was removed by filtration and the aqueous leaf extract was concentrated under vacuum to get 18% solid yield. Preliminary screening test was performed at dosages of 50, 100, 150, 200, 250, 300, 350 mg/kg bw. The plant extract was tested for restorative effect in the albino rats at the selected optimum dosage of 250 mg/kg bw and administered orally in aqueous solution.

2.3. Animals

Table 1

| Parameters                  | Group I | Group II | Group III | Group IV |
|-----------------------------|---------|----------|-----------|----------|
| Serum AST (IU/L/min/mg protein) | 68.70±4.08 | 69.12±2.05 | 107.23±8.02*** | 71.16±4.91*** |
| ALT (IU/L/min/mg protein)    | 25.36±1.75 | 24.05±0.85 | 62.76±4.95*** | 27.26±2.04*** |
| ALP (KA unit/ml)             | 11.76±0.84 | 12.25±1.05 | 32.16±2.31*** | 15.21±1.07*** |
| Liver                        |         |          |           |          |
| TBARS (nm/100 mg tissue)     | 0.52±0.03 | 0.527±0.25 | 1.72±0.129*** | 0.61±0.04*** |
| Hydroperoxide (nm/100 mg tissue) | 65.12±5.07 | 66.23±2.54 | 116.27±7.55*** | 68.21±4.84*** |
| SOD (Unit 1/mg protein)      | 7.41±0.55 | 8.23±0.94 | 2.39±0.18*** | 6.85±0.47*** |
| CAT (Unit 2/mg protein)      | 143.26±9.31 | 142.12±7.25 | 81.26±6.99*** | 132.16±9.25*** |
| GPX (Unit 3/mg protein)      | 15.21±0.95 | 11.45±1.02 | 6.71±0.46*** | 12.41±0.96*** |
| GST (Unit 4/mg protein)      | 7.21±0.49 | 8.89±1.25 | 2.81±0.20*** | 6.59±0.51*** |

*** P<0.001 as compared with group I; TBARS: content in tissue, expressed as μ moles malondialdehyde (MDA)/mg protein; SOD–U1: one unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute; CAT–U2: μ moles of hydrogen peroxide consumed per minute; GPX–U3: μ g of glutathione consumed per minute; GST–U4: μ moles of CDNB–GSH conjugate formed per minute.

Male albino rats of Wistar strain weighing around 160–180 g were purchased from Tamilnadu Veterinary and Animal Science University, Chennai. The animals were acclimatized to the laboratory conditions, fed with commercial pelleted rat chow (Hindustan Lever Ltd, Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the guidelines of institutional animal ethics committee.

2.4. Experimental protocol

The rats were randomly divided into four groups with six rats in each group. Group I: normal rats; group II: control animals which were treated with olive oil (1 mL/kg bw); group III: rats were treated with CCl₄ and olive oil mixture (1:1 V/V) at the dosage of 1 mL/kg bw i.p. for seven days to induce hepatotoxicity; group IV: the CCl₄ treated rats were subjected to the oral administration of the aqueous leaf extract of *E. alba* in an optimum dose of 250 mg/kg bw for 25 days.

2.5. Estimation of biochemical parameters

The activity levels of hepato–specific marker enzymes viz, AST, ALT in serum were estimated by the method of Reitman and Frankel[13], and the activity level of ALP in serum was estimated by the method of King[14]. The level of lipid peroxidation in liver tissue was estimated by measuring the activity of malondialdehyde and other thiolobarbituric acid reactive substances (TBARS) with thio barbituric acid (TBA)[15]. The lipid hydroperoxides in the tissue were estimated by the method of Jiang et al[16]. The liver tissue levels of enzymatic antioxidants viz. SOD, CAT, GPX, glutathione–s–transferase (GST) were estimated[17–20].

2.6. Statistical analysis

The results were expressed in mean±standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

3. Results

The activity levels of serum AST, ALT and ALP were taken
as indices for hepatotoxicity induced by CCl4. Activity levels of serum AST, ALT and ALP were significantly elevated in the CCl4 treated animals when compared to the normal group. Results were reported in Table 1. A significant increment in the levels of hepatic tissue TBARS and lipid hydroperoxides were recorded in CCl4 treated rats when compared to normal animals. The activity levels of liver antioxidant defense enzymes viz. SOD, CAT, GPX and GST were significantly decreased in the CCl4 treated animals (Table 1). The oral administration of aqueous leaf extract of *E. alba* (85%), at the optimum dosage of 250 mg/kg bw to animals caused a significant decrease in the serum levels of AST, ALT and ALP (Table 1). Treatment with *E. alba* significantly prevented the increase in the hepatic tissue TBARS and hydroperoxide levels along with a significant elevation in the activity levels of SOD, CAT, GPX and GST (Table 1).

### 4. Discussion

Measurement of the activity levels of enzymes in the body fluids is a useful monitor of overt disease of genetic predisposition of a disease state. An obvious sign of hepatic injury is leakage of cellular enzymes into serum. The enzymes AST and ALT are important cellular enzymes[21], which catalyze reversibly and bring the amino acid into the Krebs’s cycle. The ALP can transfer phosphorous group/ remove 5’-phosphate group from DNA and RNA. It will also remove phosphate group from nucleotide and protein. This enzyme is most active at alkaline pH hence the name was obtained.

In the present study, the elevated levels of serum AST, ALT and ALP in CCl4 treated animals might be due to the damage of liver tissue and/or changes in cell permeability that allow AST, ALT and ALP to leak into serum[22]. Acute hepatic injury may be cytotoxic, cholestatic or mixed type. Cytotoxic injury refers to the degeneration or necrosis of hepatic parenchymal cells[23], while cholestatic injury characterized by stagnated bile, jaundice and minimally abnormal parenchymal cells. Mixed type injury refers to hepatic injury that includes prominent cytotoxic and cholestatic components[24]. The tissue injury and increased activity levels of serum transaminase, ALP might be considered mixed hepatic cellular injury[25]. Increased activity levels of AST, ALT and ALP in the serum of the present study, reveal CCl4 induced mixed hepatic cellular type injury. Similar observations during ethanol induced hepatotoxicity were elsewhere recorded[26]. During CCl4 induced liver toxicity there will be excessive generation of free radicals. Free radicals are the ROS and are known to cause oxidative damage to a number of molecules in the cell, including membranes–lipids, proteins and nucleic acids. In the present study, the hepatic cellular injury might be due to increased oxidative stress that led to lipid peroxidation.

The levels of lipid peroxidation in the CCl4 treated rats were assessed by measuring the TBARS and hydroperoxides in the liver tissue[27]. The increased TBARS and hydroperoxide levels in the liver of CCl4 treated animals indicate enhanced lipid peroxidation leading to tissue injury. The cellular antioxidant defense mechanisms, which include scavenging activities of enzymes viz. SOD, CAT, GPX and GST play an important role in scavenging toxic intermediates of ROS. During hepatotoxicity these enzymes are structurally and functionally impaired by free radicals resulting in liver damage.

Numerous studies have shown the importance of SOD in protecting cells against oxidative stress. The decreased activity of SOD in the liver tissue of CCl4 treated animals could be due to the feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation[28]. The decreased CAT activity level in the liver tissue of CCl4 treated animals might be due to enhanced synthesis of O2·-, since oxygen radical is a powerful inhibitor of CAT[29]. The decreased activity levels of GPX in the liver tissue in the present study might be due to perturbations in normal oxidative mechanism during CCl4 ingestion. The cellular antioxidant defense enzymes viz. SOD, CAT, GPX and GST were significantly reduced in the CCl4 ingested rats. This might have allowed decreased antioxidant defense and increased oxidative stress which has thereby resulted in the tissue injury. Similar studies indicating the failure cellular antioxidant defense system during hepatotoxicity were recorded[30,31]. The activity levels of AST, ALT and ALP levels in serum are the markers to indicate the structural integrity of the liver tissue. The increased levels of these enzymes in CCl4 treated animals might be due to the leakage of enzymes into the serum[32]. The significant decrement in the levels of the ALT, AST and ALP in plant extract fed animals might be due to decreased leakage from the liver cells. This suggests that plant extract was able to repair the probable hepatic injury and/or restore the cellular permeability; thus reducing the toxic effect of CCl4 in the liver tissue. The significant depletion of levels of TBARS and hydroperoxide in the liver tissue of the plant extract fed animal group might be due to reduced lipid peroxidation and/or elevation of tissue antioxidant defense enzyme activity levels indicating that the leaf extract could reduce the free radical generation and restore free radical scavenging mechanism. The significant increment in the activity levels of SOD, CAT GPX and GST is in collaboration with the increased free radicals scavenging mechanism. Similar reduction in lipid peroxidation increased antioxidant enzyme activity levels during plant extract supplementation[33].

The above study revealed that CCl4 induced hepatotoxicity in male albino rats, was significantly reduced after being supplemented with the aqueous leaf extract of the folklore medicinal plant *E. alba*, which revealed its restorative effect.

**Conflict of interest statement**

We declare that we have no conflict of interest.
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