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Hepatoprotective activity of *Plumbago zeylanica* linn. Against carbon tetrachloride induced hepatotoxicity in rats

Iftikhar Aslam Tayubi[1†*], Ashvini Desai[2†], Inamul Hasan Madar[3,4] and Hussain Al Ssadh[5]

[1]*Faculty of Computing and Information Technology, King Abdul-Aziz University, Rabigh-21911, Saudi Arabia.*

[2]*Department of Bioinformatics, School of Biosciences and Technology, Vellore Institute of Technology, Vellore, 632014, Tamil Nadu, India.*

[3]*Department of Biotechnology & Genetic Engineering, Bharathidasan University, Tiruchirappalli, 620024, Tamil Nadu, India.*

[4]*Department of Biomedical Science and Environmental Biology, KMU-Kaohsiung Medical University, Taiwan.*

[5]*School of Biological sciences, University of Essex, Colchester, CO43SQ, United Kingdom.*

[†]*These authors made equal contribution to this work.*

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[*]**Corresponding author**: Iftikhar Aslam Tayubi, Faculty of Computing and Information Technology, King Abdul-Aziz University, Rabigh-21911, Saudi Arabia. E-mail: eislam@kau.edu.sa

Abstract

This paper evaluates the hepatoprotective activity of *Plumbago zeylanica* rhizome extracts against carbon tetrachloride (CCl4)-induced hepatotoxicity. Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl4 (0.1 ml/kg/day for 10 days). Extracts of *P. zeylanica* rhizome were administered to the experimental rats (25 mg/kg/day, p.o. for 14 days). The hepatoprotective effect of these extracts was evaluated by the assay of liver function biochemical parameters (Protein, Cholesterol, Bilirubin, Alanine amino transaminase, and Aspartate amino transaminase activities). In rhizome extract-treated animals, the toxic effect of CCl4 was controlled significantly by restoration of the levels of Protein, Cholesterol, Bilirubin Alanine amino transaminase and Aspartate amino transaminase as compared to the control cohort, which evidenced the hepatoprotective activity. Rhizome extract of *P. zeylanica* possesses significant hepatoprotective activity.

Keywords: Hepatotoxicity, carbon tetrachloride, rhizome extract, plumbagin

Introduction

Liver diseases represent a major health concern worldwide. The liver plays a vital role in drug metabolism and detoxification and as such is highly susceptible to potential damage from an enormous array of pharmaceutical and environmental chemicals [1]. The latest study reveals that 844 million people are suffering from liver diseases with 2 million deaths per year [2]. Though liver diseases are among the important diseases affecting mankind, no remedy is available to the majority of them at present [3]. Nowadays, many natural products found in vegetables, fruits, plant extracts, herbs, insects and animals are used as remedies for liver diseases and proved as a potent hepatoprotective agent [4].

Carbon tetrachloride (CCl4) is a prominent member in all the hepatotoxins and is most commonly used to induce experimental models [5]. Due to CCl4 reductive dehalogenation reaction in which cytochrome P-450 acts as a catalyst in hepatocytes, it is accepted that hepatotoxicity occurs [6]. This reaction activates the production of a few types of reactive oxygen species (ROS) [7]. These ROS further binds to lipids or proteins resulting in the formation of distinct radicals and initiates lipid peroxidation which results in membrane injury and consequently damages the liver [8].

Nowadays, the usage of synthetic drugs for treating liver diseases results in numerous side effects, therefore we should develop new drugs from medicinal plants, extracts or some other natural resources [9]. Usage of many folklore remedies for liver disorders, mainly plant products is quite common throughout India [10]. Plumbago zeylanica Linn. of family Plumbaginaceae, commonly known as white leadwort is an important medicinal plant, distributed in the tropical regions of India, tropical Africa and some parts of Oriental Asia, Australia, Yemen and Pacific islands [11]. Tilak et al. (2004) [12] reported the extracts of roots contained high amounts of polyphenols and flavonoids which inhibited lipid peroxidation. White leadwort also possesses anti-bacterial, antiplasmodial, anti-tumour, hepatoprotective, central nervous system stimulatory activity, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-cancer, anti-atherosclerotic activity etc. [10]. Studies have shown that *P.zeylanica* also possess antioxidant activity which contributes to the prevention and treatment of diseases associated with oxidative stress [12,13]. The literature study indicated that the hepatoprotective activity of rhizome of P.zeylanica has not been clinically evaluated so far. In view of this, the present study was aimed at evaluating the hepatoprotective activity of P.zeylanica against carbon tetrachloride (CCl4) induced hepatotoxicity in Wistar rats.

Materials and Methods

Plant materials and Drug preparation

Dried rhizomes of *P.zeylanica* were purchased from a traditional medical shop at Thanjavur, Tamil Nadu, India. The rhizomes were powdered through nice clothes and used as a drug.

Animals

A total of 18 pathogens free adult male albino Wistar rats (weight range: 110-130 gm) were employed for this study. The animals were obtained from the Indian Institute of Sciences, Bangalore, India and were housed in sterile cages under laminar airflow hoods in a specific pathogen-free room with a 12 hours' light and 12 hours' dark schedule and fed autoclaved chow and water ad libitum.

Chemicals

TBA, DTN B, reduced glutathione were purchased from Sigma chemicals. All other reagents used were of analytical grade.

Experimental Design

Three cohorts of rats containing six each were used for the study. Liver damage was induced in rats by administrating carbon tetrachloride (CCl4) intraperitoneal in suspension of liquid paraffin (LP) in the ratio of 1:2 V/V at the dose of 1ml CCl4/kg body weight of each animal. Cohort I served as control and received the liquid paraffin only at the dose of 1ml/kg body weight along with standard feed and water ad libitum. Cohort II served as the hepatotoxic group and received carbon tetrachloride (CCl4) in liquid paraffin suspension intraperitoneal on alternate days for a week.

Cohort III animals served as the treatment group, CCl4 was administered for two consecutive days and following 24 hours after the last injection, treatment was started by the oral administration of an aqueous suspension of *P.zeylanica* at a dose of 1g/kg body weight for 5 days.

Assessment of hepatoprotective activity

After the completion of experimental regimen, the blood was collected by puncturing the retro-orbital plexus under mild ether anesthesia. Serum was collected by allowing the blood samples to coagulate for 30 minutes at 37°C followed by centrifugation (3000 rpm for 15 minutes) and subjected for determination of biochemical parameters like total protein by the Biuret method [14], total bilirubin [15], cholesterol (Libermann and Burchard method) and the activities of serum aminotransferases (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were assayed by the method of Reitman and Frankel (1957) [16].

Results and Discussion

Liver fibrosis is a common pathological process of hepatic disease, leading to the development of irreversible cirrhosis in patients. There are various kinds of liver injuries all over the world, causing great affliction to patients. Searches for effective ways to inhibit fibrogenesis and to prevent the development of cirrhosis are of enormous significance. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost [17]. The present study evaluated the effect of P.zeylanica in CCl4 intoxicated rats by studying the biochemical parameters such as total protein, total cholesterol, total bilirubin and liver enzymes (AST and ALT).

Total protein content

The efficacy of treatment with *P.zeylanica* on the protection of the liver from CCl4 injury was evaluated by quantitative determination of the protein level in the rat model. Compared with the normal controls (6.3 gm/dl), the protein content was significantly lower in rats injected with CCl4 (4.3 gm/dl). The protein content was significantly increased in rats treated with *P.zeylanica* (5.0 gm/dl) (Figure 1).

The protein content was significantly reduced in CCl4 intoxicated rats, which reveals the severity of hepatopathy but the protein content was maintained near normal value in CCl4 rat model when treated with P.zeylanica (Figure 1). The results further elucidate the hepatoprotective effect of P.zeylanica on protein synthesis. The root powder afforded a better increase in protein which confirms its hepatic regeneration capacity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration of cells [18].

Total Cholesterol

CCl4 produced a significant decrease in the level of cholesterol (77.41 mg/dl) in the intoxicated rats compared to control (176.34 mg/dl). But CCl4 plus *P. zeylanica*-protected cohort showed a marked increase in the levels of total cholesterol (144.08 mg/dl) when compared with the CCl4 intoxicated rats and levels near control (Figure 2).

The present study shows that the level of cholesterol was elevated by CCl4 intoxication in rats (Figure 2). The hepatotoxic effects of CCl4 are largely due to production of active metabolite CCl3 by cytochrome P-450 [19]. Cholesterol reacts with free radicals and gives rise to dehydrated ketone derivatives. These products are believed to be of the significant biological outcome of CCl4 intoxication leading to lowered serum cholesterol [20].

The alteration in the total cholesterol was found to be normalized in the serum of CCl4 rats co-administered with P.zeylanica. The results show that compounds in *P.zeylanica* rhizome possess the antioxidant property and ability to scavenge free radicals produced by CCl4. Tilak et al (2004) [12] also showed that root extracts of P.zeylanica and its active ingredient plumbagin have significant antioxidant abilities.

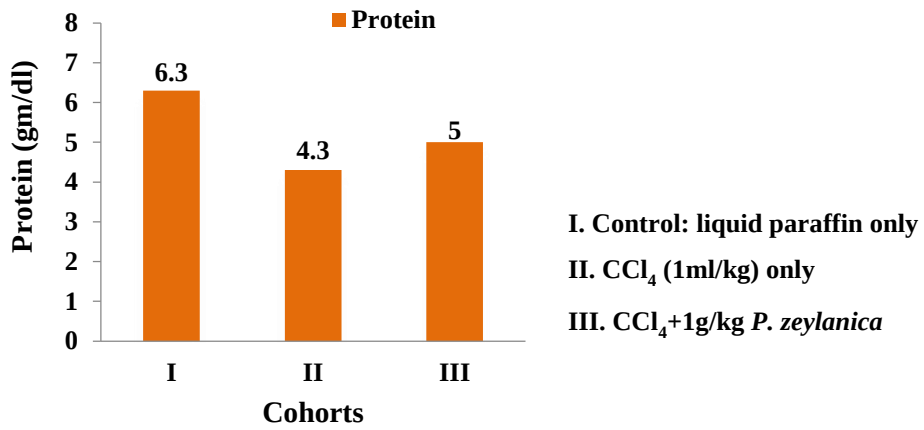

Figure 1. Effect of *P. zeylanica* on total protein in the normal and CCl_4 induced rat model

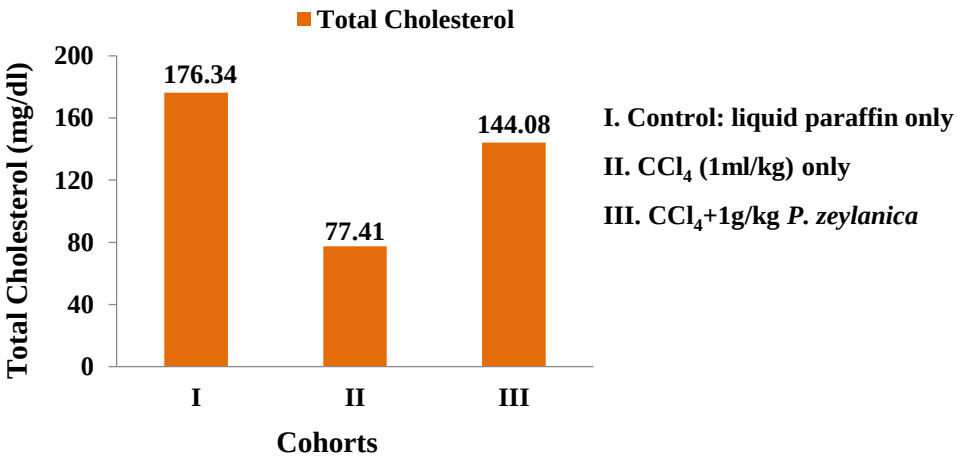

Figure 2. Effect of *P. zeylanica* on total cholesterol in the normal and CCl4 induced rat model

Total Bilirubin

The values for the level of total bilirubin in all the experimental cohorts ranged from 0.7-2.3 mg %. Treatment with *P.zeylanica* significantly decreased the level of total bilirubin (0.7 mg %) when compared to the rats intoxicated with CCl4 (2.3 mg %). The result shows that treatment cohort III values near to the controls (Figure 3).

P.zeylanica suppresses serum activities of ALT and AST in the CCl4 rat model

Biochemical analysis of serum enzymes was performed to verify the role of *P. zeylanica* in the protection of the liver from injury. It is evident that CCL4 produced a marked increase in the activities of serum ALT and AST in CCl4 intoxicated rats (Figure 4).

As compared with those in the normal controls (7.65 IU/L and 4.09 IU/L), the activities of serum ALT and AST were significantly higher in rats injected with CCl4 (12.53 IU/L and 8.83 IU/L). The activities of serum ALT and AST were significantly reduced by administration of *P.zeylanica* (6.29 IU/L and 3.46 IU/L). These results demonstrated that *P.zeylanica* protected the liver against CCl4-induced injury. The hepatotoxicity induced by CCl4 is due to its metabolite CCl3, a free radical that causes damage to liver cells. Damage to the structural integrity of liver is reflected by an increase in the levels of serum transaminases [21] because they are cytoplasmic in location and released into circular after cellular damage [22].

The present data indicate that the total bilirubin, ALT, and AST levels increased in serum following CCl4 toxicity. Hepatocyte damage is the initial factor of hepatic fibro genesis and activities of ALT and AST in serum are the most commonly used biochemical markers of liver injuries [23]. The serum levels of ALT and AST activities were markedly increased in rats injected with CCl4 [24]. Our results suggest that oral administration of *P.zeylanica* for 5 days could normalize effects induced by CCl4. This tendency of *P.zeylanica* in reducing the levels of total bilirubin (Figure 3), ALT and AST in serum (Figure 4) towards near normal level is a clear manifestation of its ant hepatotoxic effects.

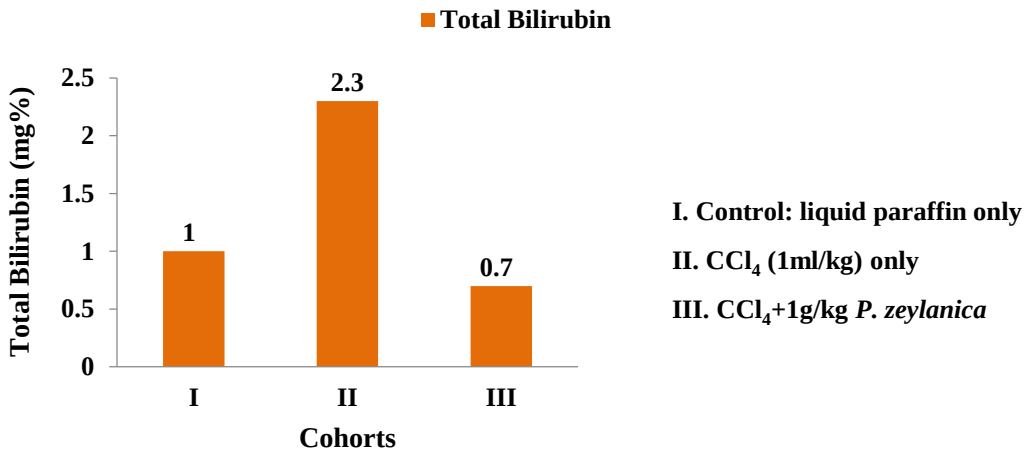

Figure 3. Effect of *P. zeylanica* on total bilirubin in the normal and CCl4 induced rat model

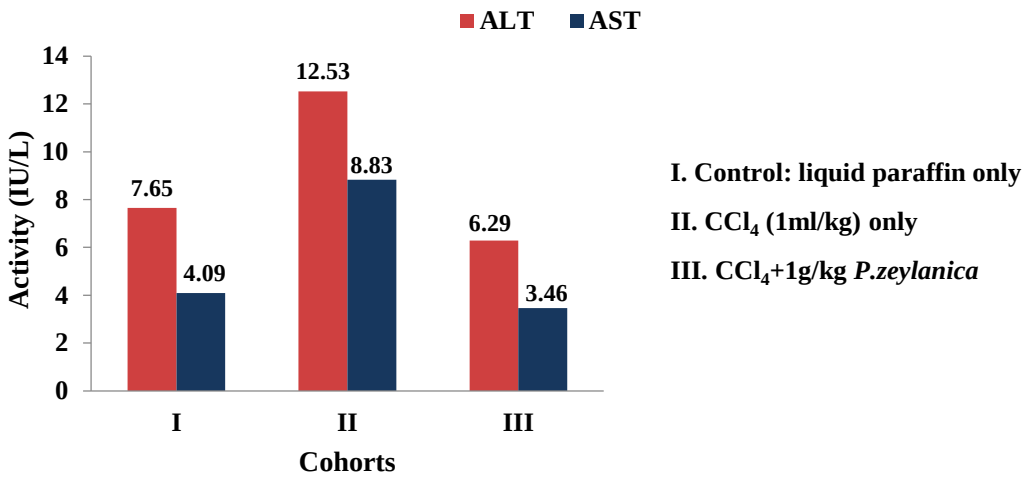

Figure 4. Effect of *P. zeylanica* on the serum activities of ALT and AST in the normal and CCl4 induced rat models

Conclusion

In conclusion, the results of the present study indicated that under the present experimental condition the root crude powder of *P. zeylanica* showed the hepatoprotective effect against carbon tetrachloride-induced liver damage in Wistar rats.

The mechanism by which *P. zeylanica* exerts its protective effect against CCl4 induced alteration may be due to the antioxidant effect of its crude root powder its active ingredient plumaged. Hence further work is necessary to elucidate the constituent responsible for hepatoprotective activity along with their mechanism of action.

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Conflict of interest

The authors declare that there is no conflict of interest.

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