International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 1, 2011

Research Article

HEPATOPROTECTIVE EFFECT OF *PLUMBAGO ZEYLANICA* ON PARACETAMOL INDUCED LIVER TOXICITY IN RATS

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Received: 16 Oct 2010, Revised and Accepted: 20 Nov 2010

ABSTRACT

Petroleum ether extract of root of *Plumbago zeylanica* was investigated for hepatoprotective activity against paracetamol induced liver damage. Various biochemical parameters were studied to evaluate the hepatoprotective activity of ethanolic extract. In serum total bilirubin, total protein, aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, γ-Glutamyl transferase, Total Cholesterol and serum triglycerides were determined to assess the effect of the extract on the paracetamol induced hepatic damage. The study was also supported by histopathology of liver sections. Results of this study revealed that the markers in the animals treated with paracetamol recorded elevated concentration indicating severe hepatic damage by paracetamol, whereas the blood samples from the animals treated with petroleum ether extract of roots showed significant reduction in the serum markers indicating the effect of the plant extract in restoring the normal functional ability of the hepatocytes. The dosage of extract of plant roots used was 300 mg/kg bodyweight of rat. The present study reveals that the petroleum ether root extract of *Plumbago zeylanica* could afford a significant protection against paracetamol-induced hepatocellular injury.

Keywords: *Plumbago zeylanica*, Petroleum ether extract of roots, Hepatoprotective activity, Paracetamol damage.

INTRODUCTION

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality[1]. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepato-protective activity[2-4].

Among the medicinal plants, *Plumbago zeylanica* (Family – Plumbaginaceae) (known as Chitrak) is a useful Indian medicinal plant which has been credited with therapeutic properties to treat several diseases. The root is blackish brown, knotted with horny fracture. Its leaves are 3 cm or so wide and about 5 cm long[5]. The root of the plant contains several bioactive chemical constituents which include plumbagin, 3-chloroplumbagin, droserone, chitranone, zeylanone, isozeylonone, plumbazeylone, coumarin, elliptinone, triterpenoids, β-sitosterol, maritinone, 2-methylnaphthazarin and anthroquinones[6]. Plumbagin is a napthoquinone and is a major component constituting about 0.03% of dry weight of the roots and is considered as the active ingredient responsible for therapeutic effects[7]. Chitrak has been used for liver problems, loose motions, chronic skin diseases, gynacological practices, dysentry, anemia and obesity. The active component plumbagin increased the fecal excretion of cholesterol and phospholipids[8]. It prevented the accumulation of cholesterol and triglycerides in liver[9]. The present study was undertaken to study the possible hepatoprotective role of petroleum ether extract of roots of *Plumbago zeylanica*.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates[10, 11]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450[12] to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI) [13]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid[14]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

MATERIALS AND METHODS

Plant materials

The roots of *Plumbago zeylanica* was collected from Adhiparasakthi Agricultural College Medicinal Park in Kalavai. The plant was identified at the Herbarium of Botany Directorate in Adhiparasakthi Agricultural College. A voucher specimen (No: Pz02) was deposited in the Botany Department of Adhiparasakthi Agricultural College, Kalavai, Tamilnadu, India.

Preparation of plant extract

The fresh dried powdered roots of *Plumbago zeylanica* were extracted (soxhlet) with petroleum ether three times at 60-80°C. All the extracts were mixed and evaporated by rotavapor. The concentrate was dissolved in minimum amount of distilled petroleum ether and the crystalline compound with a yield of 0.03% was obtained. The purity of this preparation was checked by thin layer chromatography (TLC) and gas liquid chromatography (GLC).

Chemicals

Paracetamol was purchased from, CIPLA LTD., Vill. Juddikalan, Baddi, H.P. Silymarin was supplied by Panacea Biotech Ltd, New Delhi. All other chemicals and other biochemicals used in the experiments were of analytical grade from different firms. The organic solvents were distilled before use.

Animals

Wistar Albino rats of either sex weighing between 100-200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24 ± 2^{o} under 12h light dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee.

Experimental designs

The animals were divided into 4 groups of six rats each. Group I animals served as normal control and received distilled water for

seven days. Group II animals orally received paracetamol (400 mg/kg body weight) for seven days. Group III animals received 200 mg/kg body weight of standard drug silymarin and 400 mg/kg body weight of paracetamol for seven days and served as standard control. Group IV animals received 400 mg/kg body weight of paracetamol dissolved in glucose water orally along with 300 mg/kg body weight of petroleum ether extract of roots of *Plumbago zeylanica* for seven days orally.

Sample collection

Animals of all the groups were sacrificed by cervical decapitation on eighth day. Blood sample of each group was collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37^0.

Evaluation of effect on biochemical variables

The clear serum obtained after centrifugation was used for the estimation of serum alanine amino transferase, serum aspartate amino transferase[15], alkaline phosphatase[16], gamma-glutamyl transferase[17], Lactate dehydrogenase[18], serum protein[19], serum bilirubin[20], cholesterol[21] and triglyceride[22].

Histopathology study

Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 5µ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

Results of biochemical estimations were reported as mean ± SD of six animals in each group. The data were subjected to one way ANOVA using SPSS 12.0 version (SPSS, Cary, NC, USA) followed by Bonferroni's multiple comparison tests (BMCT). The P-Value was <0.01were considered statistically significant.

RESULTS

Effect of petroleum ether root extract of *Plumbago zeylanica* on paracetamol induced liver injury in rats with reference to biochemical changes in serum are given in Table 1 and 2.

Histological profile of animals is depicted in Figure 1,2,3,4. At the end of seven days treatment blood samples of paracetamol treated animals showed significant increase in the levels of total bilirubin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase, cholesterol and triglyceride compared to the normal control group, but the total protein level decreased reflecting the liver injury caused by paracetamol; whereas blood samples from the animals treated with root extract of *Plumbago zeylanica* at the dose 300 mg/kg body weight showed significant decrease in the levels of serum markers and significant increase in the total protein to the near normal value which are comparable to the values registered in the standard drug treated group of animals, indicating the protection of hepatic cells against paracetamol damage

DISCUSSION

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification[23]. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases[24]. Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier[25, 26].

Table 1: The effect of body weight and serum protein in normal, *Plumbago zeylanica* treated , Paracetamol induced hepatic damage ,*Plumbago zeylanica* treated hepatic damage rats and Silymarin treated hepatic damage groups rats

Groups	Body weight(g)		Serum protein (g %)
	Initial (g)	Final (g)	
Normal	183	190	6.32 ± 0.92**
Paracetamol induced hepatic damage	158	150	4.16 ± 1.00**
Silymarin treated hepatic damage	173	180	5.85 ± 1.19
Plumbago zeylanica treated hepatic damage	153	160	6.12 ± 0.56**

Values are expressed as mean ± SD for six animals in each group.

*Level of significance $p < 0.05$; **Level of significance $p < 0.01$

The paracetamol-induced liver damage was treated with *Plumbago zeylanica* for seven days continuously. The following observations were obtained. Table 1. represents the bodyweight of the different groups of rats. The body weight of Paracetamol induced group II rats has been reduced comparing with group I. After 7-days administration of silymarin and *Plumbago zeylanica* on group III and group IV rats has gained the body weight almost to near normal level. The gain in body weight of group IV is compared with group III and it may be due to the regeneration of liver cells activity to near normal after the herbal treatment.

Table 2: The levels of serum bilirubin, serum cholesterol, and serum TG in normal, *Plumbago zeylanica* treated , Paracetamol induced hepatic damage ,*Plumbago zeylanica* treated hepatic damage rats and Silymarin treated hepatic damage groups rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)	γ - GT (IU/L)
Normal	92.35± 2.23*	58.33 ± 7.98*	73.67 ±6.41*	110.21± 7.16*	2.02 ± 0.09*
Paracetamol induced hepatic damage	159.98±13.23*	169.93±8.47*	131.80±12.30*	193.51±10.78*	4.82 ± 1.10*
Silymarin treated hepatic damage	101.08± 9.82**	68.83±7.65**	74.11± .35**	116.38± .58**	2.07 ± 0.85**
Plumbago zeylanica treated hepatic damage	106.00 ± 8.03*	62.00± .52**	84.12± .66**	98.69± 13.08*	2.43 ± 0.45**

Values are expressed as mean ± SD for six animals in each group.

*Level of significance $p < 0.05$; **Level of significance $p < 0.01$

AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase, LDH = Lactate dehydrogenase, γ-GT = Gamma glutamyl transferase, TG = Triglycerinde, IU/L = International units/litre,

Table 2 represents the changes in the activities of aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase γ-glutamyl transferase. In the assessment of liver damage by paracetamol the determination of enzyme levels such as aspartate transaminase and alanine transaminase is largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver[27]. Hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transminase, alanine transaminase represents 90% of total enzyme and high level of alanine transminase in the blood is better index of liver injury, but the elevated levels of enzymes are decreased to near normal levels after seven days treatment of *Plumbago zeylanica* indicates that it offered protection by preserving the structural integrity of the hepatocellular membrane against paracetamol.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure[28]. Increased level was obtained after paracetamol administration and it was brought to near normal level by *Plumbago zeylanica* treatment.

Lactate dehydrogenase is localized in the cytoplasm of cells and thus is extruded into the serum when cells are damaged or necrotic. The measurement of total lactate dehydrogenase can be useful when only a specific organ, such as the liver, is known to be involved. Lactate dehydrogenase is increased in acute necrosis of the liver. Lactate dehydrogenase is a sensitive intracellular enzyme which increases in serum is also an indication of liver cell damage[29].

γ-glutamyl transferase is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ-glutamyl transferase is generally elevated as a result of liver disease, since γ-glutamyl transferase is a hepatic microsomal enzyme. Serum γ-glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in γ-glutamyl transferase is parallel to those of amino transferases. The acute damage caused by paracetamol increased the γ-glutamyl transferase level but the same attains the normal after *Plumbago zeylanica* treatment due to its antioxidant activity.

Chronic administration of paracetamol produced a marked elevation of the serum levels of enzymes in treated animals when compared with that of the control group. Treatment with *Plumbago zeylanica* at a dose of 300 mg/kg significantly reduced the elevated levels of those enzymes. Treatment with *Plumbago zeylanica* decreased the serum levels of aspartate transaminase, alanine transaminase towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchymal cells. Effective control of alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase levels points towards an early improvement in the secretory mechanism of the hepatic cell.

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins except for the γ globulins. Hypoproteinemia is a feature of liver damage due to significant fall in protein synthesis. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the β and γ globulins as a result of production of IgG and IgM[30]. Hypoproteinemia was observed after paracetamol ingestion but the trend turns towards normal after *Plumbago zeylanica* treatment.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. Hyperbilirubinemia was observed due to excessive heme destruction and blockage of biliary tract. As a result of blockage of the biliary tract there is a mass inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged and dead hepatocytes[31]. Administration of *Plumbago zeylanica* decreased the level of bilirubin and increased the level of protein suggesting that it offered protection.

Paracetamol seems to cause impairment in lipoprotein metabolism[32] and also alterations in cholesterol metabolism. The levels of cholesterol and triglyceride were significantly increased in paracetamol treated rats, when compared to control, silymarin and *Plumbago zeylanica* treated rats. Elevation of tryglycerides level during paracetamol intoxication could be due to increased availability of free fatty acids, decreased hepatic release of lipoprotein and increased esterification of free fatty acids. Administration of *Plumbago zeylanica* significantly decreased serum lipid profile in paracetamol toxicity induced rats because of its hypolipidemic effects. *Plumbago zeylanica* supplementation enhanced esterification effect through hepatoprotective property by inhibiting the free radicals effect on liver cells.

Histological profile of control animals showed normal hepatocytes (fig.1). The section of the liver of the toxic control group of animals exhibited severe intense congestion, hydropic degeneration, pyknosis and occasional necrosis (fig.2). The liver section of the silymarin treated animals showed normal hepatic architecture with few fatty globules (fig.3). The liver section of the animals treated with root extract showed normal hepatic cords and absence of severe congestion, pyknosis and occasional necrosis (fig.4) indicating pronounced protection of hepatocytes by paracetamol induced hepatic damage.

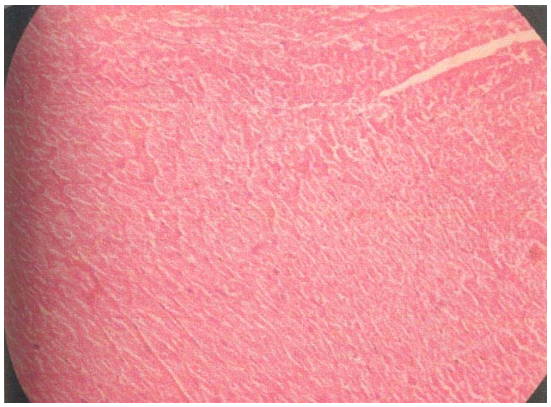

Fig. 1 (Normal)

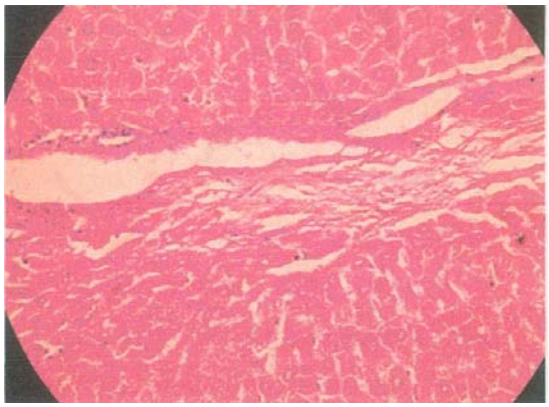

Fig. 2 (Paracetamol induced)

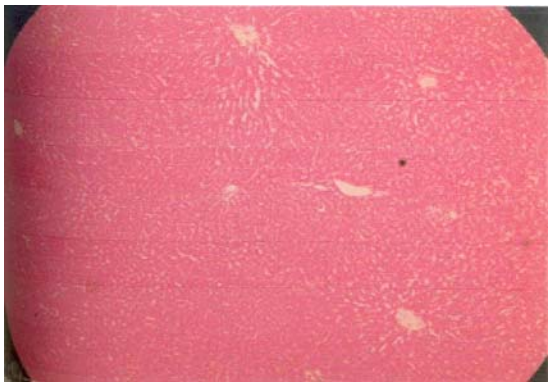

Fig. 3 (Silymarin treated)

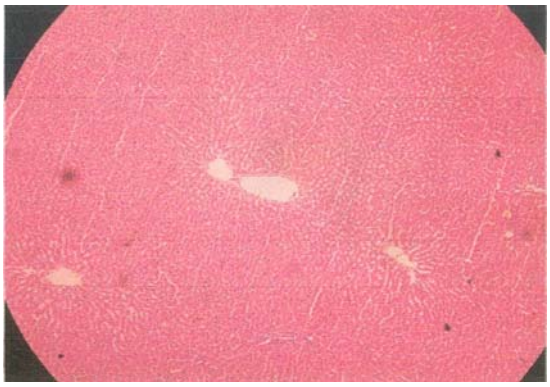

Fig.4 (*Plumbago zeylanica* treated)

Fig.1 Control Rat Liver H & E x100; Normal architecture of liver
Fig.2 PCM treated Rat Liver H & E x100; Severe congestion, hydropic degeneration, Pyknosis and occasional necrosis
Fig.3 PCM + Silymarin treated Rat Liver H & E x100; Near normal appearance of hepatocytes
Fig.4 PCM + *Plumbago zeylanica* treated Rat Liver H & E x100; Near normal appearance of hepatocytes (no severe congestion, Pyknosis and necrosis)

CONCLUSION

Several phytoconstituents *viz.*, triterpenes, sterols, zeylonone of *Plumbago zeylanica* have been found effective in the hepatoprotection against paracetamol- induced hepatic toxicity. So results of this study demonstrated that the *Plumbago zeylanica* has significant protection on paracetamol-induced hepatotoxicity.

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