



PROTECTIVE EFFECT OF *Solanum Pubescens* LINN ON CCL₄ INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Ethanol extract of *Solanum pubescens* Linn was evaluated for hepato protective and antioxidant activities in rats. The plant extract (500mg/kg/day) showed a remarkable hepatoprotective and antioxidant activity against Carbon tetrachloride (CCl₄)-induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. CCl₄ induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, LPO with a reduction of total protein, superoxide dismutase (SOD), catalase, and reduced glutathione (GSH). Treatment of rats with plant extract (500 mg/kg) significantly (P<0.01) altered serum marker enzymes and antioxidant levels to near normal against CCl₄ - treated rats. The activity of the extract at dose of 500 mg/kg was comparable to the standard drug, Silymarin (50 mg/kg, p.o.). Histopathological examination of the liver tissues supported the hepatoprotective activity of plant.

Key words: *Solanum pubescens* Linn, CCl₄, Hepatoprotective, Silymarin.

INTRODUCTION

Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from drugs, xenobiotics and oxidative stress. Certain medicinal agents when taken in overdoses and sometime even when introduced within therapeutic ranges may injure the organ. Other chemicals such as agents used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. (Achiliya *et al.*, 2003).

Among others the mechanism of action of Carbon tetrachloride (CCl₄), bromobenzene, chloroform, paracetamol, ethanol and polycyclic aromatic hydrocarbons are activated into their corresponding reactive metabolites through the action of the cytochrome P₄₅₀ system mainly located in the liver in more abundant amount than in any other organ such as lung, kidney or intestine (Sheweita *et al.*, 2001).

Solanum pubescens usually 2 or 3 m in height and 2 cm in basal diameter, but may reach 5m in height and 8cm in basal diameter. The shrub usually has a single stem at ground level, but it may branch on the lower stem. The stem bark is gray and nearly smooth with raised lenticels. The roots are white. Foliage is confined to the growing twigs (Howard, 2003).

Solanum pubescens contains a number of potentially pharmacologically active chemicals including the sapogenin steroid, chlorogenin. A related cholecalciferol, is the active ingredient in a number of commercial rodenticides. Extracts of the plant are reported to be useful in the treatment of hyperactivity colds and cough pimples, skin diseases and leprosy. Methyl caffeate, extracted from the fruit of *Solanum pubescens* shows an antidiabetic effect in streptozotocin induced diabetic rats. The present study is aimed to evaluate the hepatoprotective and antioxidant activity of ethanol extract of *Solanum pubescens* against CCl₄-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant materials

The whole plant of *Solanum pubescens* was purchased from local traditional medical shop at Thanjavur, Tamil Nadu, India.

Preparation of ethanol extract

The whole plant of *Solanum pubescens* were washed several times with distilled water to remove the traces of impurities from the plant. The plant was dried at room temperature and

coarsely powdered. The powder was extracted with 100% ethanol for 48 hours. A semi solid extract was obtained after complete

elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical analysis

Phytochemical tests were carried out on the ethanolic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofawara (1993), Trease and Evans (1989) and Harborne (1984).

Experimental design

Animals

Male albino rats of wistar strain approximately weighing 150-200g were used in this study. They were healthy animals and housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°C and 12 hours light/dark cycle throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use.

Chemicals

Nitro blue tetrazolium (NBT), Carbon tetrachloride (CCl₄), Thiobarbituric acid (TBA), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), and L ascorbic acid were purchased from Sigma Chemical Company (St.Louis,USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Body weight of the animals were recorded and they were divided in to 4 groups of 6 animals each as follows

- Group I: Normal control rats were fed with standard diet and served as a vehicle control, which received liquid paraffin at the dose of 3.0ml/Kg intraperitoneally.
- Group II: Rats were induced with hepatocellular damage by receiving suspension of CCl₄ in olive oil. (1:2v/v, 1ml of CCl₄ i.p/Kg body weight) once in every day for 7 consecutive days.
- Group III: Rats were treated with *Solanum pubescens* orally (through intragastric tube) at the dose of 500mg/Kg body weight for every day in addition to CCl₄ suspension for 7 consecutive days.

- Group IV: Standard drug as Silymarin at the dose of 200mg/Kg body weight. Once in every day for 7 consecutive days.

Collection of Samples

On completion of the experimental period, animals were anaesthetized with thiopentane sodium (50mg/Kg). The blood was collected without EDTA as anticoagulant. Serum was separated by centrifugation and used for various biochemical analysis.

Assessment of liver function

Biochemical parameters i.e., aspartate amino transferase (AST), alanine amino transferase (ALT) (Reitman and Frankel, 1957) alkaline phosphatase (ALP) (Kind and King, 1954) total bilirubin (Malloy and Evelyn, 1937) and total protein (Lowry *et al.*, 1951) were analyzed according to the reported methods. The liver was removed, morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation (LPO) (Devasagayam and Tarachand, 1987), superoxide dismutase (SOD) (Marklund and Marklund, 1974) Catalase (Sinka, 1972) and glutathione reductase (GSH) (Moron *et al.*, 1979). A portion of liver was fixed in 10% formalin for histopathological studies.

Histopathological studies

The liver tissue was fixed in 10% normal saline for 72h after which the tissues were sliced to a thickness of 2.1mm each. These were dehydrated using alcohol of graded concentration. They were further treated with paraffin wax and cast in to blocks. Sections of the tissues were cut on a microtome to 5µm. These were later attached to a slide and dried. The samples slides were viewed on

Table 2: Effect of *Solanum pubescens* on Liver marker enzymes in experimental rats.

Treatment	ALT(IU/dl)	AST(IU/dl)	ALP(IU/dl)	Bilirubin(mg%)	Protein(mg%)
Group I	23.17± 0.21	31.97± 0.51	41.36± 1.35	0.75± 0.09	6.18± 0.62
Group II	57.01 ±0.26*	60.19± 0.58*	54.04± 1.30*	1.51± 0.06*	3.52± 0.52*
Group III	23.18± 0.51	30.12 ±0.47	42.48 ±1.08	0.74± 0.06	5.78± 0.53
Group IV	24.84 ±0.49	33.18± 0.66	43.27± 0.86	0.82± 0.07	6.03 ±0.45

Values were expressed as mean ± SD for six rats in each group. * Significantly different from Group I (p≤0.05)

Analysis of LPO levels by thiobarbituric acid reaction showed a significant (P<0.05) increase in the CCl₄ treated rats. Treatment with *S.pubescens* (500 mg/kg) significantly (P<0.01) prevented

a photographic microscope to find out histological changes.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

RESULTS

The phytochemical screening of *Solanum pubescens* showed that the presence of alkaloids, terpenoids, steroids, saponin while tannins, flavonoids, glycosides were absent (Table-I).

Table 1: Phytochemical screening of *Solanum pubescens*

Phytochemicals	Results
Alkaloids	+
Glycosides	-
Tannins	-
Flavonoids	-
Terpenoids	+
Steroids	+
Saponin	+

+ indicates presence whereas – indicates absence

The effect of *S.pubescens* on serum marker enzymes is presented in Table II. The levels of serum AST, ALT, ALP, total bilirubin, were markedly elevated and that of protein decreased in CCl₄ treated animals, indicating liver damage. Administration of *S.pubescens* extract at the dose of 500 mg/kg remarkably prevented CCl₄-induced hepatotoxicity in rats (Table-II).

Table 3: Effect of *Solanum pubescens* on Antioxidant enzymes in experimental rats

Treatment	LPO (nmol/L)	Catalase (µ/ml)	SOD(U/ml)	GSH(mg/dl)
Group I	2. ±0.74	60.47± 1.81	15.24± 0.76	12.52± 1.5
Group II	15 ±1.48*	41.83± 1.25*	9.42± 0.47*	2.42± 0.39*
Group III	2.4± 0.94	57.34± 1.72	14.34± 0.71	10.87± 1.04
Group IV	2.5 ± 0.12	62.75± 1.88	16.72± 0.83	13.84± 0.35

Values were expressed as mean ± SD for six rats in each group. * Significantly different from Group I (p≤0.05)

CCl₄- treatment caused a significant (P<0.05) decrease in the level of SOD, Catalase, GSH in liver tissue when compared with control group (Table-II). The treatment of *S.pubescens* at the dose of 500 mg/kg resulted in a significant increase of SOD, Catalase, GSH when compared to CCl₄ treated rats. The liver of Silymarin treated animals also showed a significant increase in antioxidant enzymes levels compared to CCl₄ treated rats.

The histological observations basically support the results obtained from serum enzyme assays. The liver sections of CCl₄ intoxicated rats showed massive fatty changes, necrosis, ballooning degeneration, and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries. The histological architecture of liver sections of rats treated with *solanum pubescens* (500mg/Kg) showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control groups.

DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The

hepatotoxic effects of CCl₄ are largely due to its active metabolite, trichloromethyl radical (Johnson and Kroening, 1998). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of CCl₄ (Kaplowitz *et al.*, 1986). This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP, total bilirubin and decrease in protein.

The diagnosis of organ disease/damage is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of cellular damage, the intracellular concentration of the enzymes and the mass of affected tissue. The concentration of the enzyme released reflects the severity of the damage. ALT and AST are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are damaged or dies transaminases are released into blood stream, where they can be

measured they are therefore the index of liver injury (Vasudha et al., 2006).

Serum ALP, bilirubin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel and Garcipiana, 1992)

Administration of *S. pubescens* to CCl₄ intoxicated rats restored the level of ALT, AST and ALP offering the maximum hepato protection with respect to different liver marker enzymes. This confirms the liver protective effect of *Solanum pubescens*.

The study of lipid peroxidation is attracting much attention in recent years due to its role in disease processes. Membrane lipids are particularly susceptible to LPO due to the presence of polyunsaturated fatty acids. It has been implicated in the pathogenesis of a number of diseases and clinical conditions (Kale and Sitasawad, 1990). Administration of *Solanum pubescens* significantly decreased the level of LPO demonstrate the reduction of oxidative stress in *S. pubescens* and CCl₄ intoxicated rats.

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury. SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissues. The activity of this enzyme declined in CCl₄ administered rats revealed that LPO and oxidative stress elicited by CCl₄ intoxication have been nullified due to the effect of *Solanum pubescens* (Lin et al., 1998).

Catalase is a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules (Jayakumar et al., 1996). Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of *S. pubescens* increases the activities of catalase in CCl₄ induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from CCl₄ intoxication.

GSH is a major non-protein thiol in living organism, which plays a central role of co-ordinating the body's antioxidant defense process. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds such as free radicals. Glutathione status is a highly sensitive indicator of cell functionality and viability. Perturbation of GSH status of biological system has been reported to lead to serious consequences (Pastore, 2003). Decline in GSH in the liver of CCl₄ intoxicated rats, and its subsequent return towards near normally in CCl₄ and *Solanum pubescens* treated rats reveal antioxidant effect of *Solanum pubescens*. Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals (Fraga et al., 1987).

On the basis of above results, it can be concluded that ethanol extract of *Solanum pubescens* is a valuable of protection against hepatotoxicity by CCl₄ in animal model by minimizing biochemical parameters and tissue injury. The hepatoprotective activity of *Solanum pubescens* may be due to the presence of alkaloids. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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