



HEPATOPROTECTIVE POTENTIAL OF *LASIANTHUS LUCIDUS* LEAF EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN SWISS ALBINO MICE

*¹Katyayani Dutta Choudhury, ²Manabendra Dutta Choudhury, ³Mukul Kumar Baruah

¹Research scholar, Ethno-botany & Medicinal plant research laboratory, Department of Life Science & Bioinformatics, Assam University. Silchar -788011, Assam, India.

²Professor, Ethno-botany & Medicinal plant research laboratory, Department of Life Science & Bioinformatics, Assam University. Silchar -788011, Assam, India.

³Assistant Professor, Department of Botany, Cachar College, Silchar- 788001, Assam, India.

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*Correspondence for Author

Katyayani Dutta Choudhury

Research scholar, Ethno-botany & Medicinal plant research laboratory, Department of Life Science & Bioinformatics, Assam University. Silchar, Assam, India.

ABSTRACT

Lasianthus lucidus Blume is used in hepatic disorders in folk medicines. The present study was aimed to evaluate the efficacy of *L. lucidus* on the liver functions in carbon tetrachloride (CCl₄) induced injuries in Swiss albino mice. Enzymatic activities that are SGPT, SGOT, ALKP and total bilirubin and microscopic appearance of liver was used as parameter and hepato-curative studies were performed. The enzyme levels were assayed by using UV kinetic assay based on reference method of IFCC. Cytotoxicity of CCl₄ was estimated by quantifying the release of MDA. The activity of tissue antioxidant enzymes namely SOD, CAT and the level of total protein and GSH were also measured. Various leaf extracts (petrol ether, ethyl acetate, acetone and methanol) and leaf decoction pre-treatment at the dose of

200mg/kg reduced levels of SGPT, SGOT, ALKP and total bilirubin (P<0.05) previously increased by administration of CCl₄. Silymarin (25mg/kg oral dose) was used as positive control. The activity of antioxidant enzymes in CCl₄-treated group was decreased and these enzyme levels were significantly (P<0.05) increased in leaf extract and leaf decoction treated groups. Histopathological studies reveal that the concurrent administration of CCl₄ with the extract exhibited protection of liver tissue, which further evidenced the above result. The overall experimental results suggest that the biologically active phytoconstituents such as

flavonoids, glycosides alkaloids present in all the extracts and decoction of *L. lucidus* leaf, may be responsible for the significant hepatoprotective activity and the results justify the use of *L. lucidus* as a hepatoprotective agent.

Keywords: - Hepatoprotection, cytotoxicity, carbon tetrachloride, histopathology.

INTRODUCTION

The liver is the most important organ in the body. It plays a pivotal role in regulating various physiological processes. It is also involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles ^[1-2]. Any injury to it or impairment of its function may lead to many implications on one's health. The liver transforms and excretes many drugs and toxins. These substances are frequently converted to inactive forms by reactions that occur in the hepatocytes ^[3-6]. Management of liver diseases is still a challenge to modern medicine. The allopathic medicine has little to offer for the alleviation of hepatic ailments whereas the most important representatives are of phytoconstituents. Historically, plants have been used in the folk medicine to treat various diseases. Experimental works on several plants have been carried out to evaluate their efficacy against chemically induced liver toxicity ^[7-8].

Carbon tetrachloride (CCl₄) was the first toxin for which it was shown that the injury it produces is largely or entirely mediated by a free radical mechanism. Its main toxic effects are shown on the liver. Toxic levels administered to animals produce fatty accumulation in the liver due to a blockage in the synthesis of the lipoproteins that carry triglycerides away from this organ. It is believed that CCl₄ is metabolized by the P₄₅₀ system to give the trichloromethyl radical, a carbon-centred radical. Several P₄₅₀ are involved including CYP2E1, the ethanol inducible cytochrome P₄₅₀. Hence, CCl₄-induced hepatotoxicity serves as an excellent model to study the molecular, cellular and morphological changes in the liver ^[2, 9].

Lasianthus lucidus Blume (Rubiaceae) is a shrub native to tropical and subtropical regions of Asia, especially in tropical regions of China ^[10] and North East India ^[11]. There is very sporadic information available in literature about the medicinal properties of this plant despite its wide availability, however decoction of leaves have been reported to stop bleeding and applied to fever ^[12]. The genus *Lasianthus* grow almost exclusively in the understory of primary forests, with the occasional records from secondary or seriously disturbed forests or

forest edges. The genus has a great ecological significance and important component of vegetations in tropical forests of Asia^[13]. The leaves have been reported for its antimicrobial^[14] and antioxidant property^[15]. To the best of our knowledge, the hepatoprotective effect of *L. lucidus* leaves has not been documented till date. Hence, the present study is focused on evaluating the potential hepatoprotective effect of crude extracts and decoction of *L. lucidus* leaves on CCl₄-induced liver injury in Swiss albino mice.

MATERIALS AND METHODS

Plant material

The leaves of *Lasianthus lucidus* Blume were collected during month of October-November from Assam university campus, Cachar district, Assam, India and authenticated by Herbarium section of Department of Life Science and Bioinformatics, Assam University, Silchar.

Preparation of Leaf Extracts

The plant material (8.0 kg) was dried and crushed to powder and then successively extracted to exhaustion with petroleum ether (60–80°C), ethyl acetate, acetone and methanol using cold percolation method. The different extracts thus obtained were dried under reduced pressure to get the crude extract (200g), (400g), (650g) and (150g) respectively.

Preparation of Water Decoction of *L. lucidus* Leaves

Firstly, 100 g of *L. lucidus* leaves were cleaned with water to clear the dust and sand and boiled in one litre and a half of water. The water boiled for 10-15 minutes and then the mixture was taken off and drained. Then, the fluid was collected in a glass container for the usage.

Experimental Animals

Age-matched (7- to 10-week-old) pathogen free female Swiss albino mice were used to study the hepatoprotective activity of the *L. lucidus* leaf extracts. The Institution Animal Ethics Committee of Assam University, Silchar has approved the animal study for this research work. The animals were kept at 27 ± 2 °C, relative humidity 44–56% and light and dark cycles of 10 and 14 h respectively, for a week before and during the experiments. Animals were provided with standard diet (Lipton, India) and water ad libitum. The food was withdrawn 18–24 h before starting the experiment. All experiments were performed in the

morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals ^[16].

Drugs and Chemicals

Carbon tetrachloride: SD Fine Chemicals, Mumbai

Silymarin: Microlabs, Bangalore

Liquid paraffin: HiMedia Laboratories Pvt. Ltd, Mumbai

Reduced glutathione: HiMedia Laboratories Pvt. Ltd, Mumbai

All drugs and chemicals were purchased commercially and were of analytical grade.

Silymarin Dose Regimen

Silymarin tablets were obtained from a nearby clinic. Each tablet contains 500 mg of silymarin. The dose administered to the mice to induce hepatotoxicity was set as 25 mg/kg. The Silymarin was turned into a fine powder using a mortar and pestle to increase the dissolution. The powdered silymarin was suspended in pure water and was administered orally according to the body weight of mice.

Grouping of Mice and Treatments

Forty eight mice (25–30 g) were randomly divided into eight groups, each group consisting of six mice. The mice dose was calculated on the basis of surface area ratio ^[17].

Group I: Control (Pure water 10 ml/kg, oral dose)

Group II: CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Group III: Silymarin (25mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Group IV: Petrol ether extract (PE) (200mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Group V: Ethyl acetate extract (EA) (200mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Group VI: Acetone extract (AC) (200mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1; 2ml/kg, intraperitoneally)

Group VII: Methanol extract (ME) (200mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1, 2ml/kg, intraperitoneally)

Group VIII: Decoction of leaf (DC) (200mg/kg, oral dose) + CCl₄: Liquid paraffin (1:1, 2ml/kg, intraperitoneally)

Test group animals (Group IV-VIII) were administered orally a dose of 200 mg/ kg of petrol, ethyl acetate, acetone, methanol and aqueous (decoction) extracts respectively in the form of aqueous suspension once daily. The group III- VIII animals were administered simultaneously CCl₄: liquid paraffin (1:1, 2ml/kg body weight, intraperitoneally) after 30 minutes of administration of the silymarin and extracts. All the groups were treated for 5 consecutive days ^[18]. At the end of this period, animals were kept overnight fasting and were sacrificed. Blood samples were withdrawn by puncturing cardiac membrane. The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 minutes and analysed for various biochemical parameters. The liver was removed carefully after killing the animals by cervical dislocation. The livers were fixed in 10% buffered formalin and stored for determination of antioxidant enzyme levels and histopathological examinations.

Measurement of Biochemical Parameters

Blood samples were collected from heart under chloroform anesthesia and the serum was used for the assay of marker enzymes namely serum glutamate-pyruvate-transaminase (SGPT), serum glutamate-oxalate-transaminase (SGOT), alkaline phosphatase (ALKP) and bilirubin. The enzyme levels were assayed by using Ultra Violet kinetic assay based on reference method of International Federation of Clinical Chemistry (IFCC) ^[19-21]. The SGPT, SGOT and Bilirubin levels were assayed using standard kits obtained from Medsource Ozone Biomedicals Pvt. Ltd., India and that of ALKP was obtained from Synergy Bio, Quantum Biologicals Pvt. Ltd., India.

The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA) ^[22], total protein ^[23], reduced glutathione (GSH) ^[24] and antioxidant enzymes viz. Catalase (CAT) ^[25] and superoxide dismutase (SOD) ^[26] levels.

Statistical analysis

Results of the biochemical estimations are reported as mean± Standard error (SE). Total variation, present in a set of data was estimated by one way analysis of variance (ANOVA) followed by Dunnett's test using statistical package for social sciences (SPSS) version 10.0. P<0.05 was considered significant ^[27-28].

RESULTS

Biochemical Parameters

The animals treated with CCl₄ exhibited a significant ($p < 0.05$) rise in SGPT, SGOT, ALKP and bilirubin levels when compared to the control group. This were significantly ($p < 0.05$) reduced after treatment with leaf extracts and leaf decoction, which were almost similar to that of silymarin (Table I).

Table I: - Effect of *Lasianthus lucidus* leaf extracts on serum biochemical parameters against CCl₄- induced hepatotoxicity in Swiss Albino mice.

TREATMENT	SGPT (U/L)	SGOT (U/L)	ALKP (U/L)	BILIRUBIN (mg/dl)
Group I	50.63± 0.30	65.67± 0.50	105.03± 0.17	8.92± 0.23
Group II	144.33± 0.15 ^a	215.33± 2.14 ^a	156.63± 0.22 ^a	11.6± 0.18 ^b
Group III	76.24± 0.78 ^c	41.32± 0.23 ^c	76.88± 0.19 ^c	10.52± 0.18
Group IV	66.34±1.16 ^c	55.87± 0.50 ^c	47±0.28 ^c	6.13± 0.13 ^d
Group V	121.05± 0.79 ^c	65.76± 0.57 ^d	54.31±0.14 ^c	6.55± 0.09 ^c
Group VI	58.19± 0.64 ^c	45.91± 0.88 ^c	86.51±0.27 ^c	0.684± 0.01 ^c
Group VII	41.90± 4.24 ^c	171.10± 0.58 ^d	112.15± 0.28 ^c	7.85± 0.36 ^c
Group VIII	60.45± 0.57 ^c	67.84± 0.37 ^c	87.22± 0.12 ^c	7.90± 0.05 ^d

Values are expressed as mean± standard error (SE); n=6 in each group. ^aP<0.01 vs control group; ^bP<0.05 vs control group; ^cP<0.01 vs CCl₄ treated group; ^dP<0.05 vs CCl₄ treated group.

Lipid peroxidation

The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly ($p < 0.01$) higher in the CCl₄ treated group than measured in the control group. Treatment with leaf extracts and decoction decreased the elevated MDA levels. The MDA level for silymarin was also found to be significantly decreased ($p < 0.01$) (Table II).

Total Protein

Total protein level was significantly ($p < 0.05$) reduced in the CCl₄ treated group when compared to the control and were significantly elevated ($p < 0.05$) in plant extract treated groups. These were comparable with that of silymarin-treated group (Table II).

Antioxidant enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and GSH were decreased significantly ($p < 0.01$) after CCl₄ treatment and were significantly ($p < 0.01$) elevated in leaf extract treated groups (Table II).

Table II: - Effect of *Lasianthus lucidus* leaf extracts on liver malondialdehyde (MDA), total protein, glutathione (GSH) and antioxidant enzymes against CCl₄-induced hepatotoxicity in Swiss Albino mice.

TREATMENT	MDA (μmol/g tissue)	PROTEIN (μg/ml)	GSH (μmols of GSH/g wet tissue)	SOD (units/mg liver protein)	CAT (units/mg liver protein)
Group I	47.20±1.12	24.56±0.63	34.33±2.33	45.23±0.44	43.30±0.95
Group II	134.10±0.45 ^a	9.04±0.91 ^b	12.56±1.07 ^a	23.02±0.98 ^a	21.09±2.78 ^a
Group III	50.21±0.89 ^c	14.56±1.13 ^c	24.33±0.55 ^d	47.67±0.63 ^c	50.67±1.43 ^c
Group IV	56.42±0.56 ^c	19.32±0.64 ^d	74.34±0.64 ^c	56.43±2.67 ^d	67.11±1.02 ^c
Group V	75.34±2.34 ^c	17.37±0.64	65.01±1.10 ^c	64.39±0.34 ^c	50.23±0.18 ^c
Group VI	67.32±2.01 ^c	10.72±1.78	25.04±0.60 ^d	46.91±1.62 ^d	46.48±0.74 ^c
Group VII	71.27±0.53 ^c	16.21±0.25 ^d	30.13±0.73 ^c	88.03±0.90 ^c	70.34±3.01 ^c
Group VIII	85.59±0.88 ^c	11.31±3.78	29.75±0.90 ^c	39.11±0.18 ^c	41.43±0.73 ^c

Values are expressed as mean± standard error (SE); n=6 in each group. ^aP<0.01 vs control group; ^bP<0.05 vs control group; ^cP<0.01 vs CCl₄ treated group; ^dP<0.05 vs CCl₄ treated group.

Histopathology

Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. Disarrangement of normal hepatic cells with necrosis and vacuolisation are observed in CCl₄ intoxicated liver (Fig. I). The liver sections of mice treated with 200 mg/kg body weight, oral dose leaf extracts and leaf decoction of *L. lucidus* followed by CCl₄ intoxication (Fig. III, photomicrograph of methanolic extract) showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard silymarin (Fig. II), supplementing the protective effect of the test drug and the standard hepatoprotective drug.

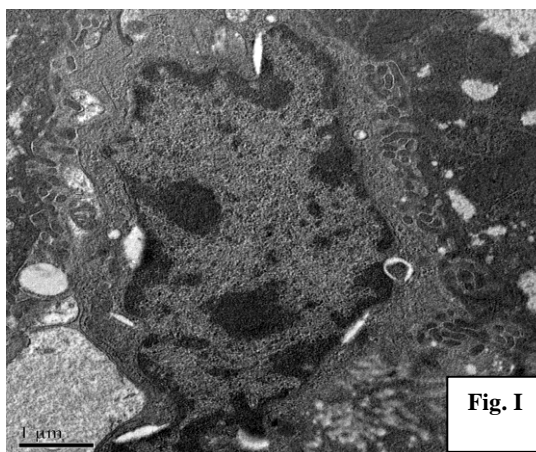


Figure I: Transmission Electro-micrograph of mice liver after CCl₄ treatment

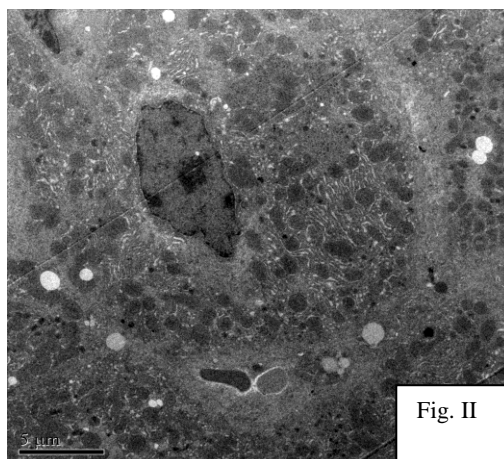


Figure II: Transmission Electro-micrograph of mice liver after CCl₄ + silymarin (25 mg/ kg) treatment

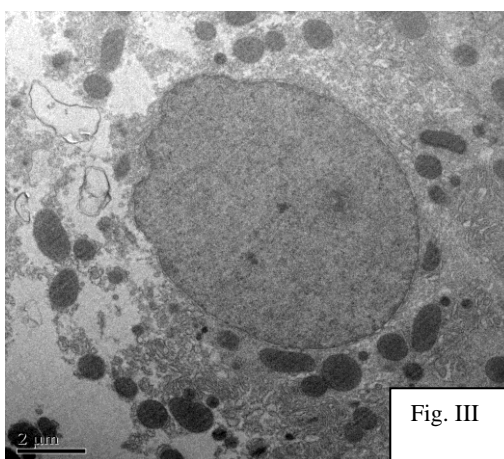


Figure III: Transmission Electro-micrograph of mice liver after CCl₄ + methanolic leaf extract (200 mg/ kg) treatment

DISCUSSION

It is well-established that CCl₄ induces hepatotoxicity by metabolic activation; therefore, it selectively causes toxicity in liver cells maintaining seminormal metabolic function. The CCl₄ is biotransformed by the cytochrome P₄₅₀ system (CYP2E1) in the endoplasmic reticulum to produce trichloro methyl free radical ($\bullet\text{CCl}_3$) when combined with cellular lipids and proteins in the presence of oxygen from trichloromethyl peroxy radical ($\bullet\text{OCCl}_3$) which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxy free radical leads to cell death^[29]. Assessment of liver damage can be made by estimating the activities of serum SGPT, SGOT, ALKP and Bilirubin originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage.

The effects of *L. lucidus* extract on liver marker enzymes and serum bilirubin content are given in Table I. The data showed that the control group demonstrated a normal range of SGPT, SGOT, ALKP and bilirubin levels, while the CCl₄-treated group showed elevated levels of SGPT, SGOT, ALKP and bilirubin, confirming that CCl₄ caused liver injury at higher doses. The elevation of cytoplasmic SGPT, SGOT and ALKP is considered an indicator for the release of enzymes from disrupted cells. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides its various normal functions, the liver excretes the breakdown product of haemoglobin, namely bilirubin, into bile. It is well known that necrotizing agents like CCl₄ produce sufficient injury to the hepatic parenchyma to cause large increases in bilirubin content ^[30]. On the other hand, the extract-treated group showed a very interesting result. Based on the Table I data, the biochemical parameters of the extract treated group were higher than those of the control group ($p < 0.05$), but it showed much lower levels of SGPT, SGOT, ALKP and bilirubin than the CCl₄-treated group, that is, the extract treatment significantly reduced the previously raised levels of SGPT, SGOT, ALKP and bilirubin in hepatotoxic mice. The decrease in the serum levels of these enzymes might be due to the presence of various phenolic and flavonoid compounds in the leaf extract that enhanced the liver's regeneration ability.

It is clear from the tabulated data (table I) that the activity of SGPT in methanolic extract (ME) group was close to its standard control group ($P = 0.002$). Acetone extract (AC) exhibited SGOT level close to that of silymarin ($P = 0.007$) and lower than the control group. PE extract lowered the ALKP level in mice as compared with the control group ($P = 0.003$) followed by ethyl acetate (EA), acetone (AC), decoction (DC) and methanol (ME) extract. In case of bilirubin the lowest value has shown by AC extract much lower than that of control group ($P = 0.002$). The results of the study show that a significant protection from toxin CCl₄ damage may be achieved mostly through all the leaf extracts studied and the leaf decoction which suggests the presence plant's protective constituents.

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation ^[31]. The result suggests that there was a dramatic increase in lipid peroxidation after CCl₄-treatment and it was inhibited by the treatment of plant extracts revealing that it exhibits potent hepatoprotective activity (table II).

Measurement of protein concentration was mainly used to calculate the level of purity of a specific protein. High doses of CCl₄ cause depletion of total proteins indicating tissue damage

which was also evidenced in this study. Treatment with CCl₄ significantly depleted GSH, CAT and SOD stores indicating that they were used for the detoxification of toxic metabolites of the drug. The leaf extracts and decoction restored the antioxidant enzyme levels significantly and reduced the CCl₄-induced oxidative injury, thus proving its antioxidant potential ^[23].

Histopathological examination of liver section of normal mice showed normal hepatic cells with cytoplasm and nucleus whereas CCl₄ treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of leaf extracts and decoction (200 mg/kg, oral dose) significantly normalized these defects in the histological architecture of the liver (Figure I-III).

CONCLUSION

From the present study, we can conclude that *L. lucidus* leaf extracts and water decoction of leaf show hepatoprotective and antioxidant activity against CCl₄ -induced hepatotoxicity. Hence these leaf extracts may act as prophylactic as well as curative drug in treating hepatotoxic conditions. Further studies need to isolate the active constituents and also to evaluate the exact mechanism of action.

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