

HEPATOPROTECTION STUDY OF LEAVES POWDER OF *AZADIRACHTA INDICA* A. JUSS.

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ABSTRACT

Liver disorders are a serious health problem. In allopathic medicinal practices reliable liver protective drugs are not available but herbs play important role in management of liver disorders. Numerical medicinal plants are used for the same in ethnomedical practices and in traditional system of medicines in India. The aim of the present work is to evaluate the effect of *Azadirachta indica* leaves powder against carbon tetrachloride (CCl₄) induced liver damage. The evaluation markers used were GOT, GPT, Alkaline phosphate, glucose, bilirubin, cholesterol and total protein. These biochemical parameters were significantly changed due to single dose of CCl₄, but the treatment of aqueous slurry of powder of leaves of *Azadirachta indica* significantly recovers all markers to normal levels. In this study silymarin was used as a standard for comparison. The observation of markers as well as Light and electron microscope photographs supports the regeneration of liver parenchyma. This proves overall promising effect against liver disorders.

Keywords: enzyme markers, hepatoprotection, *Azadirachta indica*.

INTRODUCTION

Liver disorder is one of the common thirst area declared by the Indian Council of Medical Research, New Delhi in the reviewed research on traditional medicine. In India the percentage of liver disorder is more as compared to developed countries, *Phyllanthus* species are common against such disorders. The study of the whole dry plant powder of these species has been reported.

Azadirachta Indica is a fast growing evergreen popular tree found commonly in INDIA, Africa and America. The leaves of this tree has ability to expel worms from body and used for cough, ulcers, inflammation of liver and skin diseases. It is used as purgative to treat urinary problems, tumors, piles and tooth ache. *Azadirachta Indica* is used as insect repellent¹, for skin disorders like ringworm, alopecia, eczema, urticaria, scabies, ticks and lice in animals². It is used as antifungal, antibacterial and antiviral³⁻⁶. It shows antiatherosclerotic activity⁷, antidiabetic activity^{8,9}, antimalarial activity¹⁰, antinociceptive activity¹¹, antipyretic activity¹², antiulcer activity¹³, anxiolytic activity¹⁴, cardiovascular activity¹⁵, CNS depressant activity¹⁶, hepatoprotective activity¹⁷, hypoglycaemic activity¹⁸, antiinflammatory activity¹⁹⁻²², antitumour activity²³, antifertility activity²⁴⁻²⁶, insecticidal and growth regulatory activity²⁷⁻²⁹, antiulcer activity³¹ and immunomodulatory activity³².

The present work was carried out to investigate the hepatoprotective action of *Azadirachta indica* leaves powder on CCl₄ (Carbon tetra Chloride) induced liver damaged in rats. Blood and tissue biochemical assays like ALT, AST, bilirubin, Total Protein cholesterol, Alk-PO₄, glucose etc have been studied for evaluation of hepatoprotection. From the results of these parameters it is clear that *Azadirachta indica* leaves powder gave best recovery.

MATERIALS AND METHODS

Plant material were collected from various places in Pune district, cleaned and dried at room temp in shade. They were powdered and stored in airtight containers. The powder is sieved through sieve of mesh to 85 (BSS).

The acute toxicity study of *Azadirachta indica* leaves powder was carried out on Swiss mice with a dose of 2, 4 and 6g/Kg body weight orally in the form of aqueous slurry. The exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no mortality recorded even at the highest dose level i.e. 6g/ Kg body weight and *Azadirachta indica* leaves powder had no significant toxic effects.

Dose

The dose selected for this plant powder aqueous slurry is 0.5 g/Kg body weight against CCl₄ damaged liver in rats, previously for selection of amount of dose acute toxicity study has been also done in mice. All observations are found to be normal. The daily dose regime is given in table no 1.

Animals

The animals used for study were Wister Albino rats (120-150 gm) obtained from Raj Biotech (INDIA) Pvt. Ltd, Pune 411 038. They were acclimatized for 25 days before study. They were housed in polymethane cages. Each cage housed six animals and was maintained at 28 ± 2 °C. The animals were subjected to 12 hrs cycles of light and darkness. They were fed with commercially available feed pellets (12mm) containing crude protein (min 20-21 %), crude fiber (max 4 %), calcium (1-2 %) and phosphorus (0.6 %). Animals were supplied tap water from bottles during the experiment per day and the amount food and water intake is noted³³⁻³⁶.

Parameters Observed

Blood of animals was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood Biochemical assays were determined using a STAT FAX 2000 Autoanalyser spectrophotometrically. The blood parameters observed were Alkaline Phosphates, ALT, AST (Aspartate Transferase Alanine Transferase) and Bilirubin were as tissue parameters like Glucose, total protein and Cholesterol by using Standard kits supplied by Span Diagnostics Ltd., Surat, INDIA.

Animal Grouping

Animals were grouped into five groups, each group with 12 animals 6 males and 6 females. Reversible liver

damage was induced by 0.7 ml/Kg of CCl₄ in 0.5 ml. Liquid Paraffin per animal i.p. The dose of plant powder in the form of aqueous slurry was given orally via gavages as per dose chart in Table No.1.

Gr. I served as Normal Control; Gr. II served as CCl₄ Control, Gr. III served as CCl₄ Recovery, Gr. IV served as CCl₄ + Plant Slurry (*Azadirachta indica* leaves powder) and Gr. V served as CCl₄+ silymarin (a known hepatoprotectant). The animals from all groups were sacrificed on 4th day and for of the study except the natural recovery group which was sacrificed on VIIth day after natural recovery/ regeneration of liver was initiated³⁴⁻³⁶.

Table 1: Daily Dose Regime

Day No	Group I [Normal, Control]	Group II [CCl ₄ Control]	Group III [CCl ₄ Recovery]	Group IV [CCl ₄ + Plant material]	Group V [CCl ₄ + Silymarin]
1	0.5cc liq. Paraffin And 2 cc d/w orally	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. and 2cc d/w orally	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. and 2cc d/w orally	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p. and 0.5gm/kg plant material in 2cc d/w orally	0.7cc/kg CCl ₄ in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w orally
2	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.5gm/kg plant material in 2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally
3	2cc d/w orally	2cc d/w orally	2cc d/w orally	0.5gm/kg plant material in 2cc d/w orally	0.007gm/kg Silymarin in 2cc d/w orally
4	Sacrifice	Sacrifice	2cc d/w orally	Sacrifice	Sacrifice
5	-	-	2cc d/w orally	-	-
6	-	-	2cc d/w orally	-	-
7	-	-	Sacrifice	-	-

Note: The above dosage is for an individual animal of the group.

The number of animals in each group = 6 males and 6 females.

i.p. = intra peritoneal. ; d/w = distilled water; liq. paraffin = liquid paraffin.

RESULTS AND DISCUSSION

Liver damage due to CCl₄

Literature survey reveals that CTC causes hepatic injury and is a well-known liver toxin. CCl₄ has direct destructive effect on membranes of the hepatocyte and on consequent interface with cellular metabolism and transport. It damages the membranes of the hepatocyte causing leakage of the enzymes present in the cell. This results in elevation of the levels of plasma transaminases.

It leads to fat decomposition in the liver due to blockage of secretion of hepatic triglycerides into plasma. The toxicity of CCl₄ depends upon the cleavage of C-Cl bond to generate a trichloro methyl- a free radical (CCl₃O₂). This cleavage occurs in the endoplasmic reticulum and is mediated by the cytochrome P-450 mixed function oxidase system. The product of the cleavage binds irreversibly to hepatic proteins and lipids. The metabolism of CCl₄ releases CCl₃ a free radical, which initiates per oxidation and cleavage of fatty acids in the membranes. The CCl₄ derived free radicals initiates the process of peroxidations by attacking Methylene Bridge of unsaturated fatty acid side chains of microsomal lipids. This results in early morphological alteration of endoplasmic reticulum and eventually to ultimate cell death through of series of changes listed below besides as yet underlined pathways

like loss of activity of P450 xenobiotic metabolizing system, loss of glucose-b- phosphatase activity, loss of protein synthesis, loss of capacity of liver to form and excrete VLDL (Very Low Density Lipoproteins). Alterations in these parameters are used to monitor the course and extent of CCl₄ induced liver damage.

A single dose of CCl₄ leads to centrilobular necrosis and fatty liver. Within a few minutes, there is injury to the endoplasmic reticulum leading to functional defects of the Hepatocyte and multiple biochemical manifestations of hepatic injury. Irrespective of the route of administrations it leads to centrilobular necrosis and steatosis. Biochemical changes in the blood reflect injury. Serum enzyme levels increase with cytoplasmic enzyme reaching their peak within 12 hrs. Mitochondria enzymes reach their peak within 36 hrs. Enzymes common to both mitochondria and cytoplasm reach their peak around 24 hrs.

CTC causes accumulations of fat in the liver especially by interfering with the transfer of triglycerides from the liver into the plasma. Many clinical conditions that cause an increase in cholesterol levels also cause increase in triglycerides enzymes sensitive to cytotoxic injury are serum glytamic pyruvic transaminase (SGPT) now called Alanine amino transferase (ALT) and serum glytamic oxaloacetic transferase (SGOT) now known as Aspartate

amino transferase (AST). Aspartate and Alanine amino transferases are present in high concentration in liver. Due to hepatocyte necrosis or abdominal membrane permeability, these enzymes are released from the cells and their levels in the blood increase. ALT is a sensitive indicator to acute liver damage and elevation of this enzyme in no hepatic disease is unusual. Alkaline phosphatase, although is not a liver specific enzyme, the liver is major source of this enzyme. Also the levels of this enzyme increase in cholestasis, elevated serum gamma-glutamyl transpeptidase levels appear to be indicative of diseases of the liver, biliary tract and pancreases. Bilirubin levels in blood also increase in liver diseases. (Cirrhosis and hepatitis).

The results obtained from blood biochemical parameters are given in table no 3. In clinical chemistry AST, ALT, values showed significant changes. All the values were higher than those of the control animals. Similar observations were noted in bilirubin and cholesterol.

Dosage

- A reversible damage was induced in rat liver by administering low concentration of CCl₄. The liver damage was induced by an intraperitoneally (i.p.) injection of CCl₄ (0.7 cm³/kg body wt) liquid Paraffin to each animal of group II to V
- An i.p injection of 0.5 cm³ of liquid Paraffin was given to each animal from Gr. I as sham treatment.

- A dose of 0.5 g/kg body wt of sieved *Azadirachta indica* leaves powder suspended in 2cc/dist water was administered orally to each rat of Gr. IV
- A dose of 0.007-g/kg-body wt of silymarin (Silybon tablets manufactured by Ranbaxy lab. Ltd. India) suspended in 2CC of DW was administered orally to each rat of group V this dose is equivalent to the prescribed human dose of Silybon tablets.

The normal control group I, CCl₄ cont. Gr. II CCl₄ natural recovery group III animals were administered 2 cc D/W as show treatment except the plant powder. The oral dosing was done using the gavage. The animals were first given CCl₄ inj. Intraperitoneally the oral dose of the drug.

The animals from Gr. I, II, IV and V were sacrificed at 72 hrs after CCl₄ liver administration (period of maximum liver damage)³³⁻³⁵ and the animals from Gr. III were sacrificed on seventh day of the study.

General Observations

Animals from all groups showed no abnormal behavior. Food and water consumptions: The food consumptions of animals from CCl₄ control, CCl₄ and plant treated and CCl₄ and silymarin group decreased significantly. The CCl₄ recovery group animals showed significant decrease up to the fourth day of the treatment, and then they showed an increase. This indicates that the animals are recovering from the toxicity induced by the CCl₄ similar observations were noted with the trends in water consumption by treated animals.

Table 2: Effect of *Azadirachta indica* leaves powder on body weight

Groups	Body weights of rats in grams						
	1 st Day	2 nd Day	3 rd Day	4 th Day	5 th Day	6 th day	7 th Day
Group I Normal Control	142.5 ± 3.8	143.8 ± 5.2	144.23 ± 3.6	146.10 ± 4.6	SACRIFICE	-	-
Group II CCl ₄ Control	130.2 ± 1.2	128.4 ± 2.2	126.2 ± 1.2	130.7 ± 3.2	SACRIFICE	-	-
Group III CCl ₄ Recovery	144.3 ± 2.7	143.7 ± 4.2	144.5 ± 2.7	145.1 ± 2.3	146.3 ± 2.3	147.3 ± 1.8	SACRIFICE
Group IV CCl ₄ +Plant Mat.	122.2 ± 4.1	123.5 ± 1.9	124.2 ± 3.2	125.1 ± 4.6	SACRIFICE	-	-
Group V CCl ₄ +Silymarin Control	133.7 ± 3.2	132.5 ± 3.7	132.4 ± 4.2	136.9 ± 3.5	SACRIFICE	-	-

Table 3: Effect of *Azadirachta indica* leaves powder on Blood Biochemical Parameters

Groups	Bilirubin (B)	GOT (B)	GPT (B)	Alk-PO ₄ (B)	Glucose (mg/dl) (B)	cholesterol (mg/dl) (B)	Total protein (B)
Group I	0.51 ± 0.02	145.50 ± 12.20	96.224 ± 4.24	142.44 ± 3.01	130.51 ± 02.23	98.11 ± 6.14	6.32 ± 0.2
Group II CC	0.80 ± 0.03	215.10 ± 1.10	183.3 ± 1.14	153.11 ± 04.00	160.17 ± 3.32	119.28 ± 1.40	4.01 ± 5.20
Group III CR	0.72 ± 0.02	190.00 ± 2.22	164.43 ± 2.61	162.21 ± 6.69	195.30 ± 2.23	120.54 ± 3.2	5.8 ± 1.35
Group IV CP	0.53 ± 0.01	146.12 ± 05.25	102.45 ± 2.50	139.66 ± 3.10	152.16 ± 3.44	109.22 ± 3.42	5.8 ± 1.42
Group V CS	0.52 ± 0.12	160.29 ± 5.20	173.24 ± 3.24	145.22 ± 4.30	140.9 ± 4.47	88.61 ± 2.30	6.12 ± 0.30

Biochemical parameters

CCl₄ treatment caused significant increase in plasma ALT, AST levels. These levels were not significantly recovering after natural recovery phase. The observations were competent in both the male and female animals. The plant treatment caused significant reduction in ALT and AST levels in both in male and female rats. CCl₄ treatment caused accumulation of cholesterol and the plasma levels of cholesterol were high in treated animals both in CCl₄ and CCl₄ recovery groups. *Azadirachta indica* leaves powder treatment significantly reduced cholesterol in all rats.

Plasma levels of bilirubin significantly increased after treatment, in CCl₄ control group and CCl₄ recovery groups the levels were marginally reduced for group IV and V.

Plasma levels of triglycerides increased significantly after CCl₄ treatment. These levels remain high even after natural recovery or CCl₄ treatment but plant slurry treatment showed significant reduction in triglycerides levels in female rats significantly.

Plant slurry treatment caused significant reduction in cholesterol in all rats. The tissue cholesterol levels reduced

after natural and Silymarin treatment CCl₄ treatment causes classical fatty liver as indicated by significant increase in tissue cholesterol CCl₄ treatment significantly increased plasma gamma GT levels in all treated animals. The levels decreased after plant slurry and silymarin treatment.

Total tissue protein significantly increased after CCl₄ treatment. These levels significantly decreased after natural recovery and silymarin treatment. Plant slurry treatment caused marginal reduction in total tissue proteins in rats.

The liver of the rats after combined treatment of CCl₄ and *Azadirachta indica* leaves powder (Fig.4) shows mild congestion in some of the sinusoids. The dilation of sinusoids is evident in the centrilobular areas. The vacuolation seen after CCl₄ treatment is significantly absent.

The liver of the rats after combined treatment of CCl₄ and Silymarin shows some regions of recovery. The dilation of sinusoids is evident in the centrilobular areas. The vacuolation seen after CCl₄ treatment is absent (Fig.5).

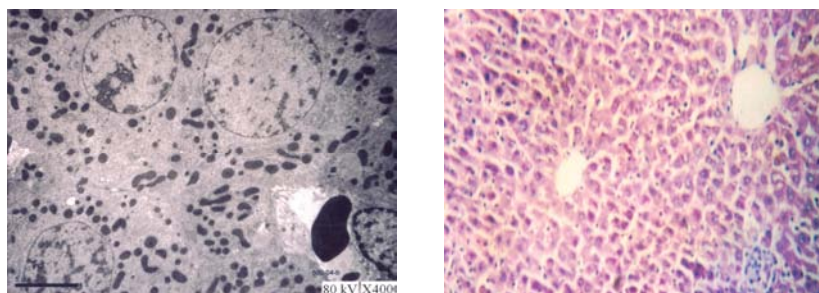


Figure 1: Electron and light micrograph of normal control group

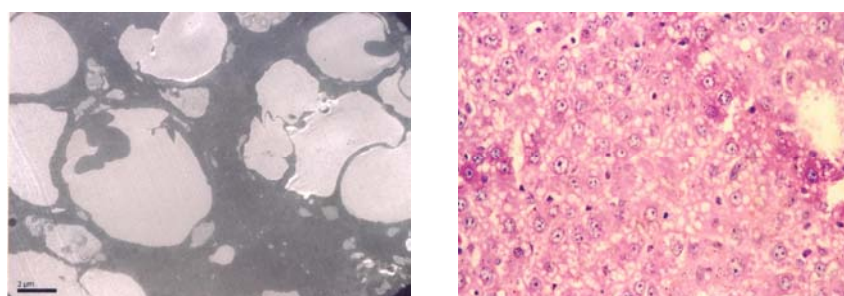


Figure 2: Electron and light micrograph of rat liver after CCl₄ treatment showing necrosis

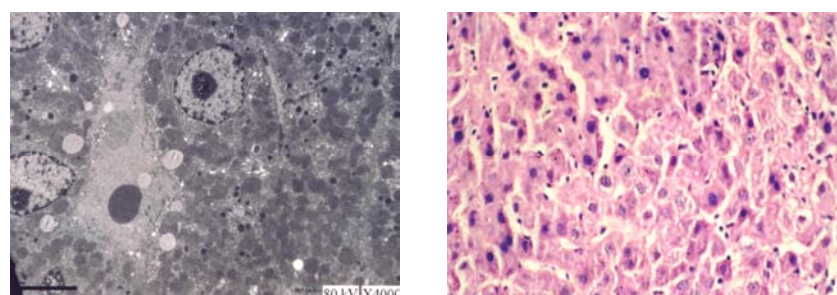


Figure 3: Electron and light micrograph of rat liver after CCl₄ natural recovery

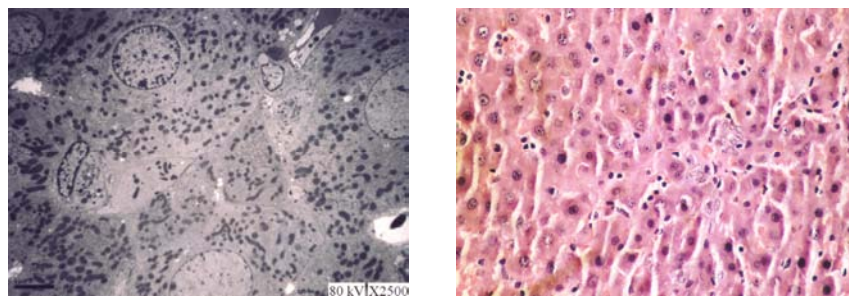


Figure 4: Electron and light micrograph of rat liver treated with CCl_4 and *Azadirachta indica* leaves powder

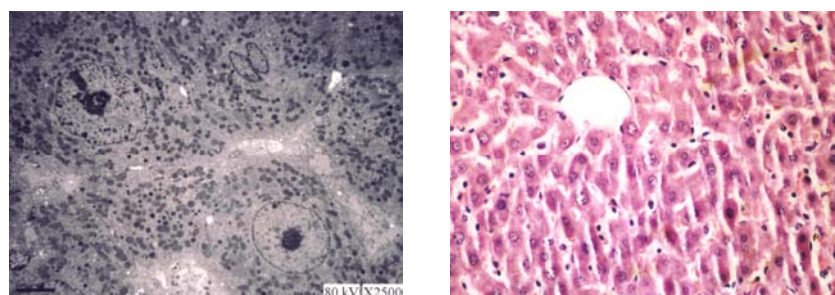


Figure 5: Electron and light micrograph of rat liver treated with CCl_4 and Silymarin

CONCLUSION

Under light microscope the liver shows distinct centrilobular necrosis after CCl_4 treatment. The hepatocytes in the acinus are vacuolated with distinct dilatation of sinusoids. After treatment of *Azadirachta indica* leaves powder, CCl_4 damage is recovered. The hepatocytes also show some signs of the recovery. The study thus clearly indicates the intrinsic hepatotoxic effect of the plant.

The present investigation therefore adequately proves that *Azadirachta indica* leaves powder is an effective hepatoprotective agent at the dose (0.50 g kg^{-1}) used in the present investigation. The plant slurry impairs normal liver function inducing distinct toxic changes in hepatocytes. This is the dose which show maximum hepatoprotective action against CCl_4 induced liver toxicity. The study reiterates the importance of standardization while formulating herbal based formulation. The overall results are very interesting, since it was demonstrated that *Azadirachta indica* leaves powder indeed has a high potential in healing liver parenchyma and regeneration of liver cells. Thus it may act even in humans as potent liver tonic.

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