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Hepatoprotective action of *Terminalia belerica* on CCl_4 induced hepatic disorders

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ABSTRACT

The importance of fruit powder of Terminalia belerica was investigated for its hepatoxicity effect in Wistar Albino rats against CCl_4 induced hepatic damage. Oral administration of 1 gm/kg body weight of powder of Terminalia belerica fruit recovered the CCl_4 induced liver damage. The elevated levels of tissue and blood biochemical markers were reported. The administration of Terminalia belerica plant material recovered the enzyme levels like normal rats. The biochemical observations were supplemented by histoapthological examination of liver. The results of blood and tissue biochemical parameters like Aspartate aminotramferase, Alanine aminottramferase, Alkaline phosphatase, etc reveled that Terminalia belerica fruit powder could regenerates liver cells and offered protection against CCl_4 induced hepatic damage. The observation of markers as well as Light and electron microscope photographs supports the regeneration process of liver parenchyma.

Keywords: *Terminalia belerica*, biochemical markers, hepatoxicity, liver parenchyma.

INTRODUCTION

Terminalia belerica is one of the known medicinal herbs of India that nourishes the lungs, throat, voice, eyes and hair. It expels accumulations in the digestive, urinary, and respiratory tracts. It is unique in being both laxative and astringent, so it purges the bowels, while simultaneously toning the tissues of the digestive tract. It provides strength to the tissues of the sense organs. It is used as Anthelmintic, antiseptic, astringent, expectorant, laxative, lithotriptic, rejuvenative, tonic. The fruit is one among the triphala of ayurveda. It is useful in asthma, biliousness, bronchitis, inflammations, sore throat, and treating the diseases of eyes, nose, heart and bladder.

Liver has an important place in toxicology by virtue of its function, both qualitatively and quantitatively. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment of its functions may lead to many implications on one's health. Management of liver diseases is still a challenge to the modern

medicine [1]. The modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representatives are of phytoconstituents [2].

Many organs in body account for any toxic action, liver is one of them. Present investigation attempts to reveal the efficacy of *Terminalia belerica* fruit powder in the form of aqueous slurry against CCl_4 induced biochemical and histopathological changes in liver of Albino Wister rats.

MATERIALS AND METHODS

The fruits of *Terminalia belerica* were collected from Avsari Forest Park, Avsari, Ambegaon, PUNE, cleaned and dried at room temp in shade. They were powdered, sieved through sieve of mesh to 85 (BSS) and stored in airtight containers with all specifications.

Dose

The dose selected for *Terminalia belerica* fruit powder in the form of aqueous slurry is 1gm/Kg body weight against CCl_4 damaged liver in rats, preciously for selection of amount of dose acute toxicity study has been carried out in mice where all observations were found to be normal.[3, 4]

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 15 days before study. They were housed in polymethane cages. Each cage housed six animals and was maintained at 25 ± 2^0C. 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 noted [5, 6].

Parameters Observed

Blood of animals was collected by cardiac puncture under light ether anesthesia during sacrifice. Blood Biochemical assays were determined using a CHEMITO SPECTRASCAN UV 2700 spectrophotometer. The blood parameters observed were GPT(ALT), GOT(AST), Cholesterol, Bilirubin, Triglecerides and √GT were as tissue biochemical parameters like Gycogen, T. Protein, Cholesterol, DNA, and RNA. This was done by using Standard kits supplied by Span Diagnostics Ltd., Surat, INDIA.

Animal Grouping

Animals were grouped into five groups. Each group consists of 12 animals 6 males and 6 females. Reversible liver damage was induced by giving dose of 0.7 ml/Kg of CCl_4 in 0.5 ml. Liquid Paraffin per animal i.p. [7, 8]. The dose of *Terminalia belerica* fruit powder in the form of aqueous slurry was given orally via gavages as per dose chart in Table I.

The animals from all groups were sacrificed on IV^{th} day and for of the study except the natural recovery group which was sacrificed on VII^{th} day after natural recovery of liver was initiated.

Table I: Daily Doses Regime

Days	Group I Normal control	Group II CCl_4. control	Group III CCl_4 treated natural recovery	Group lV CCl_4 + plant slurry treated	GroupV Silymarin treated
1	0.5cc liq. Paraffin & 2 cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p.& 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p. & 1 gm/kg slurry of plant material in 2cc d/w oral	0.7cc/kg CCl_4 in 0.5cc liq. Paraffin i.p., 0.007gm/kg Silymarin in 2cc d/w oral
2	2cc d/w oral	2cc d/w oral	2cc d/w oral	1 gm/kg slurry of plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
3	2cc d/w oral	2cc d/w oral	2cc d/w oral	1 gm/kg slurry of plant material in 2cc d/w oral	0.007gm/kg Silymarin in 2cc d/w oral
4	Sacrifice	Sacrifice	2cc d/w oral	Sacrifice	Sacrifice
5	-	-	2cc d/w oral	-	-
6	-	-	2cc d/w oral	-	-
7	-	-	Sacrifice	-	-

All dosages are for each individual animal in the group.
The number of animals in each group 12 (6 males + 6 females).
i.p. : intraperitoneal.
d/w : Distilled Water.

Gr. I served as Normal Control; Gr. II served as CCl_4 Control, Gr. III served as CCl_4 Recovery, Gr. IV served as CCl_4 + Terminalia belerica fruit powder in the form of aqueous slurry and Gr. V served as CCl_4+ Silymarin (a known hepatoprotectant).

RESULTS AND DISCUSSION

Liver damage due to CCl_4

Literature survey reveals that CTC causes hepatic injury and is a well-known liver toxin. CCl_4 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 tramaminases [9, 10].

It leads to fat decomposition in the liver due to blockage of secretion of hepatic triglycerides into plasma. The toxicity of CCl_4 depends upon the cleavage of C-Cl bond to generate a trichloro methyl a free radical (CCl_3O_2). This cleavage occurs in the endoplasmic reticulum and is mediated by the cytochrome P-450 mixed function oxidase system. The product of the clevage binds irreversibly to hepatic proteins and lipids. The metabolism of CCl_4 releases CCl_3 a free radical, which initiates per oxidation and clevage of fatty acids in the membranes. The CCl_4 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 series of changes listed below besides as yet underlined pathways like loss of activity of P-450 xenobiotics 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 CCl_4 induced liver damage [10].

A single dose of CCl_4 leads to centrilobular necrosis and fatty liver. Within a few minutes, there is injury to the endoplasmic reticulum lending 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 park 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 causes an increase in cholesterol levels also cause increase in triglycerides enzymes sensitive to cytotomic injury are serum glytamic pyruvic transaminase (SGPT) now called Alanine amino transferase(ALT) and serum glytamic oxaloacetic transferase (SGOT) now known as Asparatate amino transferase(AST). Asparatate 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 hepatic disease is unusual. Alkaline phophatase, 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 increases in liver disease. (Cirrhosis and hepatitis). The Tissue Biochemical Parameters for all Groups are given in table II.

Table II: Tissue Biochemical Parameters For All Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
Gycogen	20.55±1.01	20.40±0.21	22.30±0.88	18.5±0.73	17.50±74
T. Protein	8.4±0.55	20.20±0.21	10.5±0.54	8.1±0.22	12.1±0.52
Cholesterol	1.6±1.22	2.30±1.20	1.90±1.30	1.5±1.16	2.8±1.24
DNA	0.5±0.22	0.45±0.21	0.90±0.24	0.48±0.25	0.7±0.14
RNA	2.4±1.02	4.90±1.10	3.75±1.50	2.50±1.20	6.50±1.15

Table III: Blood Biochemical Parameters For All Groups

Parameter	Gr.I	Gr.II	Gr.III	Gr.IV	Gr.V
GPT(ALT)	75.60±1.10	70.08±1.2.	61.20±1.22	77.30±1.20	76.50±1.21
GOT(AST)	144.00±0.55	146.20±0.54	148.30±0.58	145.20±1.00	156.84±0.23
Cholesterol	75.60±1.40	82.40±1.42	75.40±1.50	78.50±1.40	69.50±1.44
Bilirubin	0.58±1.24	1.21±1.54	0.64±1.58	0.68±1.20	0.65±1.35
Triglecerides	124.50±1.50	130.00±1.45	94.80±1.52	129.501.21	134.50±1.42
√GT	18.30±1.10	41.20±1.00	33.40±1.22	22.60±1.20	24.80±1.40

The results obtained from blood biochemical parameters are given in table III. 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

1. A reversible damage was induced in rat liver by administering low concentration of CCl_4. The liver damage was induced by an intraperitonial (i.p.) injection of CCl_4 (0.7 cm^3/kg body wt) liquid Paraffin to each animal of group II to V

2. An i.p injection of 0.5 cm^3 of liquid Paraffin was given to each animal from Gr.I as sham treatment.
3. A dose of 1g/kg body wt of sieved *Terminalia belerica* fruit powder suspended in 2cc distilled water was administered orally to each rat of Gr. IV
4. A dose of 0.007g/kg-body wt of silymarin (Silybon tablets manufactured by Ranbaxy lab. Ltd. India) suspended in 2CC of distilled water was administered orally to each rat of group V this dose is equivalent to the prescribed human dose of Silybon tablets.
5. The Normal Control Gr.I, CCl_4 Control Gr.II, CCl_4 Natural Recovery Gr.III animals were administered 2 cc D/W as sham treatment except the plant powder.
6. The oral dosing was done by using the gavage number 12. The animals were first given CCl_4 injection intraperitonially and the dose of the drug orally.
7. The animals from Gr. I, II, IV and V were sacrificed at 72 hrs after CCl_4 liver administration (period of maximum liver damage) and the animals from Gr. III were sacrificed on seventh day of the study).

General Observations

Animals from all groups showed normal behavior in food and water consumptions. The food consumptions of animals from CCl_4 control, CCl_4+ *Terminalia belerica* fruit powder in the form of aqueous slurry and CCl_4+ silymarin group decreased significantly. The CCl_4 recovery group animals showed significant decrease up to the fourth day of the treatment, and then they showed slight increase. This indicates that the animals are recovering from the toxicity induced by the CCl_4. Similar observations were noted with the trends in water consumption by treated animals. The plant material treated animals' shows increase in food and water consumption.

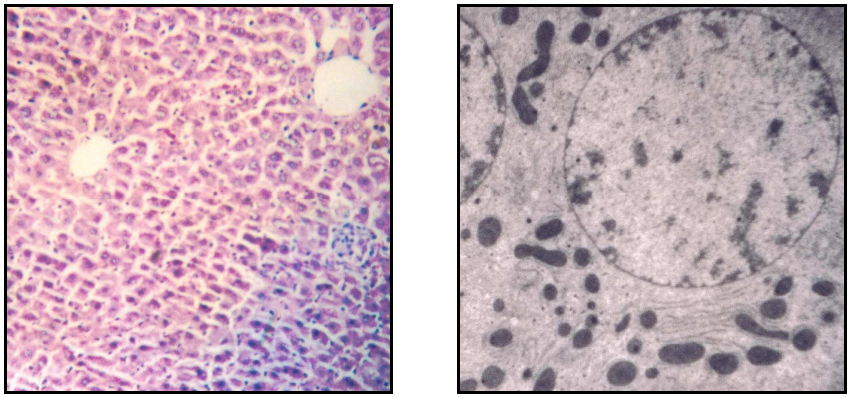

FIG. I : Light and electron micrograph of normal rat liver

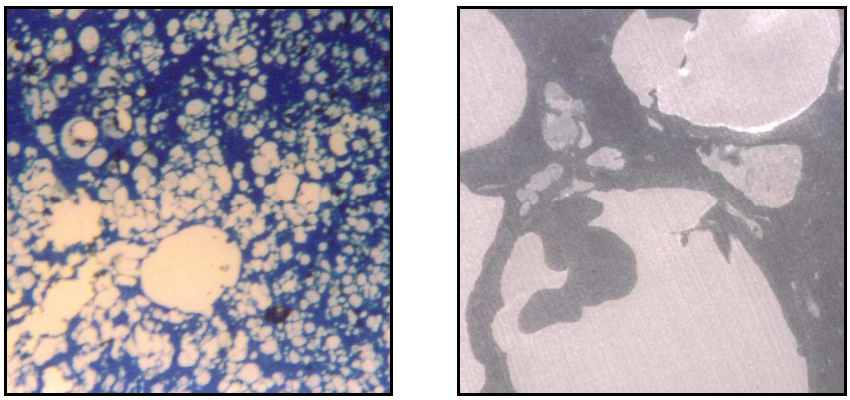

Fig. II: light and electron micrograph of rat liver after ccl_4 treatment

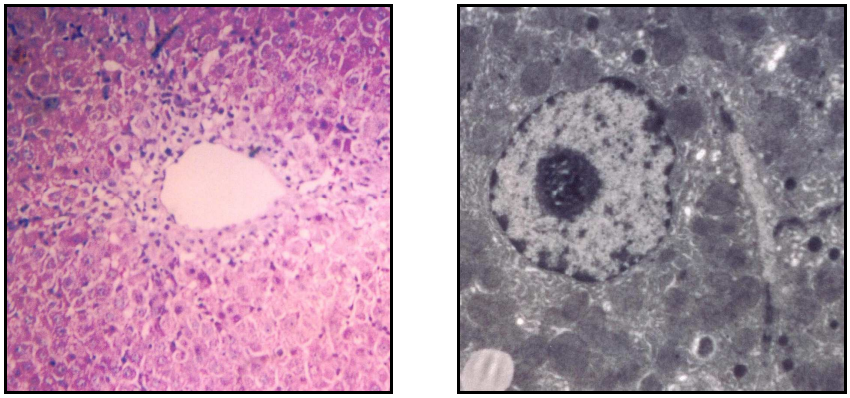

Fig. III: light and electron micrograph of rat liver after natural recovery

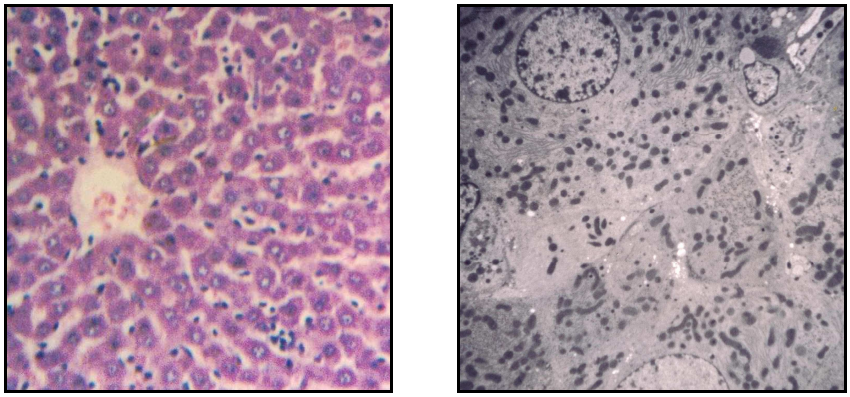

Fig.IV: Light and Electron Micrograph Of Rat Liver Treated With Ccl_4 And Plant Material

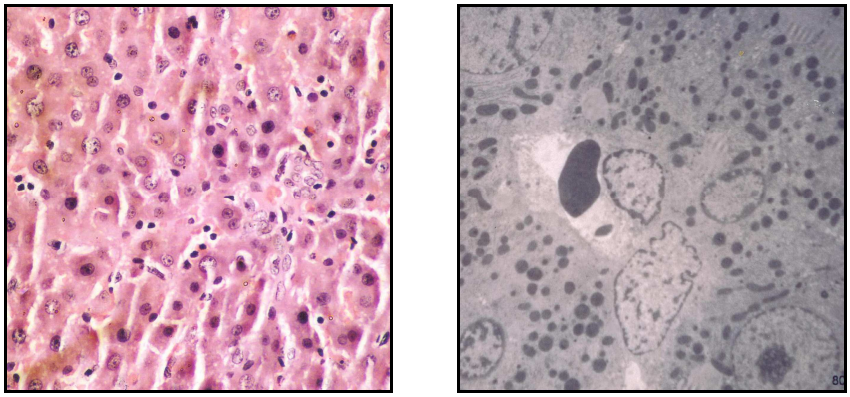

Fig.V: Light and Electron Micrograph Of Rat Liver Treated With Ccl_4 And Silymarin

Histopathology of liver of the normal control rats showed prominent central vein and normal arrangement of hepatic cell (Fig. I). CCl_4 treated rats showed various degrees of pathological changes starting from centrilobular necrosis of hepatic cells to central lobular fatty degeneration (Fig. II). The natural recovery group shows some initial signs of recovery indicating slight recovery (Fig III). Liver section of rats treated with *Terminalia belerica* fruit powder in the form of aqueous slurry showed significant regeneration against CCl_4 induced liver damage (Fig IV). The sections of liver taken from the rats treated with standard drug Silymarin showed the hepatic architecture, which was slightly better than the natural recovery group. (Fig. V).

Biochemical Parameters

CCl_4 treatment caused significant increase in plasma ALT, AST levels. There 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. CCl_4 treatment caused accumulation of cholesterol and the plasma levels of cholesterol were high in treated animals both in CCl_4 and CCl_4 recovery groups. *Terminalia belerica* fruit powder in the form of aqueous slurry treatment significantly reduced cholesterol in all rats. Plasma levels of bilirubin significantly increased after treatment of CCl_4 control group and CCl_4 recovery groups the levels were marginally reduced. Plasma levels of triglycerides increased significantly after CCl_4 treatment. The plant material treatment showed significant reduction in triglycerides levels in rats. Plant material treatment also caused significant reduction in cholesterol. The tissue cholesterol levels reduced after natural and Silymarin treatment CCl_4 treatment causes classical fatty liver as indicated by significant increase in tissue cholesterol CCl_4 treatment significantly increased plasma gama GT levels in all treated animals. The levels decreased after plant slurry and silymarin treatment.

CONCLUSION

The aim of the present study is to examine the effect of orally administrated 1gm/kg body weight powder of fruit of *Terminalia belerica* in experimental liver injury caused by CCl_4. The elevated levels of blood and tissues biochemical marker indicates induction of hepatic damage. The hepatic markers accessed by reversal of majority of altered parameters. In present study Silymarin was used as standard drug for comparison. The observations of “Group I” were matching with “Group IV” than all other groups. The combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity play important role in regeneration of liver cells.

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