

# In-vitro Antioxidant and In-Vivo Hepatoprotective Activity of Ethenolic Extract of *Tectona grandis* Bark Against CCl<sub>4</sub> Induced Liver Injury in Rats

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## ABSTRACT

**Objectives:** The systematic screening of *Tectona grandis* bark with the purpose of discovering new bioactive compounds as a hepatoprotective agent and to establish the scientific basis for the therapeutic actions of traditional plant medicines. **Methods:** *Tectona grandis* bark ethenolic extract was studied for the hepatoprotective activity against CCl<sub>4</sub> induced liver injury in rats. Serum enzymes level, total bilirubin and histopathological study of liver were performed. This extract's DPPH radical scavenging potential was also studied. **Results:** Oral administration of ethenolic extract of *Tectona grandis* bark (200 mg/kg) exhibited significant reduction ( $p < 0.05$ ) in CCl<sub>4</sub>-induced increased levels of SGPT, SGOT, ALP and bilirubin (Total) concentration. Treatment with Liv 52 syrup also reversed the hepatotoxicity significantly ( $p < 0.05$ ). Histopathological studies also provided supportive evidence for biochemical analysis. This extract also showed better activity in quenching DPPH radical. **Conclusion:** *Tectona grandis* bark ethenolic extract shown to have hepatoprotective and antioxidant action due to presence of quinones and tannin like phytoconstituents.

**Key Words:** *Tectona grandis*, Hepatotoxicity, Antioxidant, CCl<sub>4</sub> induced hepatopathy, Histopathology, Quinones.

## INTRODUCTION

Liver plays a pivotal role in metabolism, secretion and storage. Any injury to liver can result in many disorders ranging from transient elevation in liver enzymes to life threatening liver cirrhosis and hepatic failure. The common causative agents of liver injuries are toxic chemicals (e.g. CCl<sub>4</sub>, aflatoxin etc.), therapeutic drugs (e.g., antibiotics, anti-tubercular drugs etc), alcohol and microbial agents (e.g. hepatitis virus, leptospira, malarial parasites).<sup>1</sup>

The role of free radical reactions in disease pathology is well established. It suggests that these reactions are necessary for normal metabolism but can be detrimental to health as well including outcome of various diseases like diabetes, immunosuppression, neurodegenerative diseases and others.<sup>2</sup> Free radicals lead to cellular necrosis, which is implicated in some membrane pathophysiological conditions, including atherosclerosis, rheumatoid arthritis as well as toxicity of many xenobiotics.<sup>3</sup>

Liver diseases remain a serious health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals.<sup>4</sup> Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India.<sup>5</sup> Many plant species have been utilized as traditional

medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

*Tectona grandis* Linn (Verbenaceae) tree commonly known as Sagvan tree, found throughout the India. It is a huge tree, bark ash colored. The wood has a characteristic aromatic odor. The roots are useful in anuria. The bark is useful in bronchitis, hyperacidity, diabetes, leprosy and skin diseases. The flowers are useful in leprosy, skin diseases, burning sensation and diabetes. Leaves are useful in inflammation, leprosy, in skin diseases<sup>6</sup> wound healing<sup>7</sup>, diabetes.<sup>8,9</sup> The Ethenolic extract of this plant is used in the treatment of anemia.<sup>10</sup>

The literature screened in the process of the proposed work indicates that the selected plant contain classes of chemical constituents which have shown antioxidant activity. Literature survey revealed that *Tectona grandis* bark ethenolic extract has no scientific claims for hepatoprotective and antioxidant activity. Phytochemical and pharmacological investigations of this plant may yield useful information and material for better management for preventing the production of the free radicals and diabetes.

## MATERIALS AND METHODS

### Animals

Healthy adult male wistar albino rats weighing between 170-200 gm were used for the Hepatoprotective studies, whereas wistar albino rats of either sex were used for determination of acute

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toxicity study. The animals were housed in groups of 5 per cage with free access to commercial rat pallet diet (Lipton India Ltd., Mumbai, India) and water ad libitum. The animal room was maintained at 25°C ± 2°C with timed lighting on from 6 am to 6 pm and relative air humidity of 30 to 60%. The Institutional Animal Ethics Committee (CPCSEA/1/15/2007) approved the study.

## Chemicals

All chemicals and solvents used were of analytical grade from Merck Ltd., Mumbai, India and Sigma Aldrich Co., USA. Liv 52 syrup was obtained from Himalaya Drug Company, India.

## Collection of plant material

The bark of *Tectona grandis* Linn was collected from local areas of Kolhapur (Maharashtra) & Belgaum (Karnataka) India. The specimen was authenticated from Dr.S.R.Yadav, Prof., Dept. of Botany, Shivaji University, Kolhapur (Maharashtra) India. The voucher specimen (AMCOP/Pharm/07/19) was retained in the Herbarium of Department of Pharmacognosy, Ashokrao Mane College of Pharmacy, Peth Vadgaon (Maharashtra) India.

## Preparation of plant extracts

The collected plant material was washed thoroughly in water, chopped, shade dried at room temperature, reduced to a coarse powder in a mechanical grinder and passed through a 40 # sieve for desired particle size. The powder obtained was subjected for the extraction, with 95% ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure and dried. The yield of *Tectona grandis* bark ethenolic extract was 4.3 % (w/w). The obtained extract was stored in a refrigerator at 2-8°C until usage.

## Preliminary phytochemical investigations

Preliminary phytochemical investigation revealed the presence of Lapachol-a naphthaquinone (root)<sup>11</sup>, naphtho and anthraquinone derivatives (heart wood)<sup>12</sup>, terpenoids and tannins (Leaves) in *Tectona grandis* plant.

## Experimental design

Screening of *Tectona grandis* bark ethenolic extract for Hepatoprotective and antioxidant action was done in rats.

## Acute toxicity study

Determination of LD<sub>50</sub> for extracts is done by OECD guidelines for fixing the dose for biological evaluation. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The LD<sub>50</sub> of the extract as per OECD guidelines 2001, falls under 5mg, 50 mg, 300 mg and 2000 mg/kg bw with no signs of acute toxicity at respective doses. The biological evaluation of extract is carried out at 1/10 doses of LD<sub>50</sub><sup>13</sup>

## Hepatoprotective activity

Hepatoprotective activity was carried out by using albino rats. The animals were divided into four groups of six rats in each. 1% Gum acacia suspension was given to groups I & II as a vehicle for 10 days by oral route. Liv. 52 administered as a standard drug to group III at a dose of 1ml /kg by oral route up to 10 days and IV<sup>th</sup> group received *Tectona grandis* bark ethenolic extract (200 mg/kg by oral route up to 10 days).

Except group I (control group), all remaining groups were received Carbon tetrachloride at a dose of 0.7ml/kg, on 3, 6 & 10th day by intraperitoneal route. On 10 th day, 1 hr after last dose of Carbon tetrachloride, animals were sacrificed by cervical dislocation & the blood was collected from the carotid artery, serum is separated & used

for estimation of biochemical parameters such as SGPT, SGOT, ALP & Total Bilirubin. Liver was excised, quickly fixed in 10% formalin and then fixed in bovine solution and further histopathological study was done for observation of architectural changes.<sup>14</sup>

## *In-vitro* antioxidant –DPPH free radical scavenging activity<sup>15</sup>

The free radical scavenging activity of *Tectona grandis* bark ethenolic extract was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). For DPPH assay, the method of Blois was adopted. The capacity of *Tectona grandis* bark ethenolic solvent extract to scavenge the lipid-soluble DPPH radical was monitored at an absorbance of 517 nm. Ethenolic bark extract (1 ml) of *Tectona grandis*, at different concentration was allowed to react with DPPH. Thirty minutes later, the absorbance was measured at 517 nm. The percentage inhibition of absorbance was calculated for each concentration relative to a blank absorbance using the spectrophotometer. The DPPH scavenging capacity of the extracts is compared with that of BHT (Butylated hydroxytoluene). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All determinations are carried out at least three times, and in triplicate. IC<sub>50</sub> value in the tested compound is, the concentration required to scavenge 50% DPPH free radical. Percentage inhibition was calculated as DPPH radical scavenging activity.

$$\text{DPPH radical Scavenging effect (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100$$

Where, Abs control is the absorbance of initial conc. of DPPH radical; Abs sample is the absorbance of DPPH radical + sample Extract / standard.

## Statistical analysis

Values are presented as mean ± S.E.M. Statistical difference between treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the "Stat" statistics computer program. A difference in the mean values of  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### Acute toxicity study

Acute toxicity study revealed no mortality or any toxic reactions with oral administration of ethenolic extract of bark of *Tectona grandis* even at the highest dose (2000 mg/kg). The biological evaluation of extract is carried out at 1/10 doses of LD<sub>50</sub><sup>13</sup>

### Hepatoprotective activity study

Rats subjected to CCl<sub>4</sub> only, developed significant hepatocellular damage as evident from significant increase in serum activities of GPT, GOT, ALP and Total bilirubin concentration as compared to normal control group, which has been used as reliable marker of hepatotoxicity. Oral administration of ethenolic extract of *Tectona grandis* bark (200 mg/kg, p.o) exhibited significant reduction ( $p < 0.05$ ) in CCl<sub>4</sub>-induced increase in levels of GPT, GOT, ALP and bilirubin (Total) concentration. Treatment with Liv 52 syrup also reversed the hepatotoxicity significantly ( $p < 0.05$ ) (Table 1).

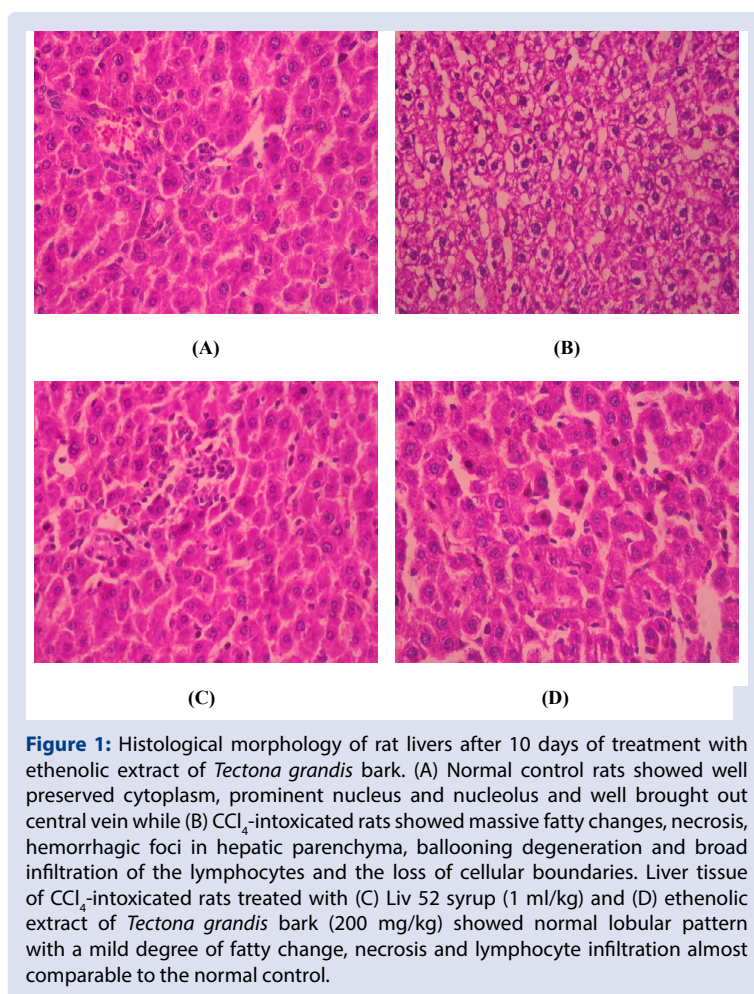
### Histopathological studies

Histopathological studies also provided supportive evidence for biochemical analysis (Figure 1). Histology of the liver section of normal control animals showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (A). The liver sections of CCl<sub>4</sub>-intoxicated rats showed massive fatty changes, necrosis, hemorrhagic foci in hepatic

**Table 1: Effect of *Tectona grandis* bark ethenolic extract on the Serum enzymes and total Bilirubin in CCl<sub>4</sub>-induced hepatotoxic rats after 10 days of treatment.**

Exp. Group (n= 6)	Treatment	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	TOTAL BILIRUBIN (mg/dl)
I	Normal control (1% gum acacia)	131.0 ± 20.5*	86.00 ± 17.1**	161.0 ± 15.2**	0.700 ± 0.07**
II	CCl <sub>4</sub> control	217.0 ± 28.7	340.0 ± 21.0	385.0 ± 27.3	2.123 ± 0.1
III	LIV 52 syrup	140.0 ± 19.1*	182.0 ± 5.1**	219.0 ± 12.2**	0.800 ± 0.1**
IV	<i>Tectona grandis</i> bark ethenolic extract (200 mg/kg)	160.0 ± 7.4*	173.0 ± 34.4**	323.3 ± 24.5*	0.900 ± 0.1**

\*P < 0.05 & \*\*P < 0.01 Significant compared to CCl<sub>4</sub> control, n= no of animals in each group



parenchyma, ballooning degeneration and broad infiltration of the lymphocytes and the loss of cellular boundaries (B). The histological architecture of liver sections of rats treated with ethenolic extract of *Tectona grandis* bark (200 mg/kg) and standard Liv 52 syrup (1 ml / kg) showed normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration almost comparable to the normal control (C and D).

### *In-vitro* antioxidant –DPPH free radical scavenging activity

Several concentrations ranging from 10-1000 µg /ml of the ethenolic extract of bark of *Tectona grandis* tested for their antioxidant activity by DPPH model. It has been observed that free radicals were scavenged by

the *Tectona grandis* bark ethenolic extract in a concentration dependent manner in this DPPH assay (Table 2).The ethenolic extract of bark of *Tectona grandis* showed DPPH radical scavenging activity with an IC<sub>50</sub> value of 211 µg /ml when compared with Standard BHT (Butylated hydroxytoluene) IC<sub>50</sub> value of 107 µg /ml.

### DISCUSSION/ CONCLUSION

Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. These are gaining popularity because of several advantages such as often fewer side effects, better patient tolerance, relatively less expensive and acceptance due to long history of use. Plants are often less prone to the emergence of drug resistance.

**Table 2: DPPH scavenging activity of Ethenolic extract of *Tectona grandis* bark.**

Concentration (µg /ml)	DPPH scavenging (% inhibition)	
	Ethenolic <i>Tectona grandis</i> bark extract	BHT (Butylated hydroxytoluene)
10	7.95	20.07
50	20.01	38.17
100	33.93	47.71
250	52.68	90.25
500	60.83	97.21
1000	79.52	95.22
IC <sub>50</sub>	238µg /ml	107 µg /ml

Data represents mean ± S.E.M. of triplicate analysis.

The 1, 1-diphenyl -2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds. Plants provide a rich source of antioxidants, which include tocopherols, Vit.C, phenolic compounds, carotenoids<sup>16</sup>, flavonoids, terpenoids, anthraquinones, steroids, strychnine and eugenol alkaloids.<sup>17</sup> From the present results, it may be postulated that *Tectona grandis* bark ethenolic extract reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principals, so it can be concluded that the *Tectona grandis* bark ethenolic extract has potent in vitro antioxidant potential which is attributed due to the presence of quinones, terpenoids and tannins like constituents present therein.

Damage of liver cell is reflected by an increase in the levels of hepatospecific enzymes; these are cytoplasmic and are released in to circulation after cellular damage. In this study significant increase in the total bilirubin content and in the SGOT, SGPT and ALP activities in the CCl<sub>4</sub> treated group could be taken as an index of liver damage. Treatment with *Tectona grandis* bark ethenolic extract inhibited CCl<sub>4</sub> induced increase in total bilirubin and SGOT, SGPT and ALP activities as compared with CCl<sub>4</sub> treated group (Figure 1).<sup>18</sup>

The mechanism of hepatic damage by CCl<sub>4</sub> is well documented. CCl<sub>4</sub> is metabolized by CYP 450 enzyme system to trichlormethyl radical (CCl<sub>3</sub>). This in turn reacts with molecular oxygen and gets converted to trichloromethyl peroxy radical. This radical forms covalent bonds with sulfhydryl group of several membrane molecules like GSH leading to their depletion and causes lipid peroxidation. The lipid peroxidation initiates a cascade of reactions leading to tissue necrosis.<sup>19</sup>

Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream. Alkaline phosphatase is a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ. Alkaline phosphatases, although not a liver specific enzymes, the liver is the major source of this enzyme. The level of this enzyme increases in cholestasis. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release.<sup>20</sup>

Ethenolic extract of *Tectona grandis* bark has significantly scavenged reactive oxygen species as indicated in Table 2. Similarly the test extract significantly reduced the elevated serum biochemical markers of hepatic injury (Table 1). It is apparent from the present results that the antioxidant property of ethenolic extract of *Tectona grandis* bark prevented the formation of trichloromethyl peroxy radical thereby reducing tissue damage. This is further confirmed by the histopathological study. Therefore, the hepatoprotective activity of ethenolic extract of *Tectona grandis* bark may be due to its antioxidant potential. Since there are reports that the plants containing quinones possess antioxidant

properties so this hepatoprotective and antioxidant properties of the test plant may be attributed to the presence quinines.<sup>21</sup>

From these results it may be concluded that the *Tectona grandis* bark ethenolic extract shown to have hepatoprotective and antioxidant action. It is conceivable that antioxidant/ free radical scavenging activity of *Tectona grandis* bark ethenolic extract is one of the mechanism associated with hepatoprotective effect. The other mechanism is presence of quinones and tannins in ethenolic extracts of *Tectona grandis* bark significantly reduced the activities of SGPT, SGOT, ALP and Total Bilirubin enzymes as compared to that of toxicant rats. However, the extract should further be subjected to bioactivity-guided drug discovery to isolate the lead compound responsible for antidiabetic activity and possible mechanisms(s) of action.

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