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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF TECTONA GRANDIS SEEDS BY USING CCL_4-INDUCED HEPATIC INJURY IN RATS.

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ABSTRACT
Liver failure can result from any type of liver disorder, including viral hepatitis (most commonly hepatitis B), cirrhosis, and liver damage from alcohol or drugs such as acetaminophen. Herbal drugs play a significant role in the regeneration of liver cells and acceleration of healing process and hence management of many liver disorders. Based on the fact that a tremendous number of plants could be considered as a gold mine for discovery of hepatoprotective agents, we launched our study in a trial to investigate the hepatoprotective activity of *Tectona grandis* seeds by using CCl_4-induced hepatic injury in rats. In the preliminary phytochemical studies confirmed the presence of carbohydrates, Phenolic compounds like tannins, flavonoids, and saponins.CCl4 treated group showed significant increase in the levels of AST, ALT and ALP as compared to that of vehicle treated normal control rats. The PTG 400 mg/kg, and Silymarin 50 mg/kg showed significant and equipotent ($P<0.01$) reduction in elevated levels of these enzymes and showed hepatoprotective effect. PTG 200 mg/kg was effective to reduce AST levels only whereas PTG 100 mg/kg was not significant at all. The result of the present study clearly indicates that the petroleum extract of *Tectona Grandis* Linn seed possesses potent hepatoprotective activity against CCl_4 induced hepatotoxicity, which claims its traditional use as a hepatoprotective agent.

KEYWORDS: Hepatotoxicity, *Tectona grandis*, CCl_4, AST, ALT and ALP.

INTRODUCTION
Liver is one of the important organ of the body which plays a major role in the metabolism of proteins, carbohydrates, lipids. It is also having wide range of functions including detoxification, storage of glycogen, vitamin A, D and B_{12}, production of several coagulation factors, growth factors (IGF-1), hormones (angiotensinogen) and biochemicals necessary for digestion (bile).[1] Liver failure can result from any type of liver disorder, including viral hepatitis (most commonly hepatitis B), cirrhosis and liver damage from alcohol or drugs such as acetaminophen. Modern food styles, excessive medications and exposure to pollutants besides many other factors have led to many serious diseases including liver damage.[2] Production of reactive oxygen species is considered as a crucial factor leading to oxidative damage of tissues by releasing free radicals.[3] CCl_4 is a potent hepatoxin producing centrilobular hepatic necrosis which is widely used for animal models of liver fibrosis. It has been reported that CYP450 in rat liver activates CCl_4 to a trichloromethyl free radical (CCl_3 and/or CCl_3OO), and stimulates Kupffer cells to produce ROS, such as O_2^-, H_2O_2 and OH, which damage the liver.[4, 5, 6]

Herbal drugs play a significant role in the regeneration of liver cells and acceleration of healing process and hence management of many liver disorders.[3] One of the comprehensive examples is silymarin isolated from Silybum marianum. Based on the fact that a tremendous number of plants could be considered as a gold mine for discovery of hepatoprotective agents, we launched our study in a trial to investigate the hepatoprotective activity of *Tectona grandis* seeds by using CCl_4-induced hepatic injury in rats.

MATERIALS AND METHODS
Plant material
Commercially available dry seeds of *Tectona grandis* Linn., were purchased in the bulk quantity from local market and authenticated and extracts are prepared as follows by the botanist from the Agriculture College, Pune.

Preparation of Petroleum ether extract[7]
Tectona grandis dried seeds were charged to extractor along with petroleum ether. It was extracted by heating the mass for 2-3 hours, in a closed system by re-pumping the extract to the herb bed. This process was repeated. The extracts obtained were then combined and filtered.

They were then concentrated under vacuum. The extract was then dried in a drier unit and further powdered in a multimill to a fine mesh size. Extract was then sieved using a Sifter to make uniform particle size.

Preliminary phytochemical investigations

The preliminary phytochemical investigations were carried out with Petroleum ether extracts of seeds of *Tectona Grandis* for qualitative identification of phytochemical constituents present with each extract and tests were carried out by following standard methods.[7, 8, 9] All the chemicals and reagents used were of analytical grade. The results are compiled in Table-01.

Determination of acute toxicity (LD_{50})[10, 11]

The acute toxicity of Petroleum ether extracts of seeds of Tectona Grandis was determined by using female albino mice (20-30g) those maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment according to up and down procedure (OECD guideline no. 425) method of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (short term) toxicity. Based on the drug short term profile the dose of the next animals were determined as per as OECD guideline 425. All the animals were observed for long term toxicity (14 days) and then 1/5th, 1/10th, 1/25th, 1/50th of the lethal dose was taken as effective dose ED_{50}.

Determination of hepatoprotective activity

Evaluation of hepatoprotective activity using Carbon tetrachloride (CCl_4) model[12]

Albino rats weighing between 150-200gm each group containing six animals will be divided in six groups. The the treatment schedule for 14 days as follows.

Group A - Normal Control (vehicle treated, p.o)
Group B - Toxicant (CCl_4 3 ml/kg, s.c.)
Group C - Standard (Silymarin 50mg/kg, p.o)
Group D – Petroleum ether extracts of seeds of *Tectona Grandis* (100 mg/kg, p.o) + after 1hr Toxicant (CCl_4 3 ml/kg, s.c.)
Group E - Petroleum ether extracts of seeds of *Tectona Grandis* (200 mg/kg, p.o) + after 1hr Toxicant (CCl_4 3 ml/kg, s.c.)
Group F - Petroleum ether extracts of seeds of *Tectona Grandis* (400 mg/kg, p.o) + after 1hr Toxicant (CCl_4 3 ml/kg, s.c.)

On 15th day, blood is collected for estimation of the liver biomarker enzymes. On an equivalent day, the liver is removed and keeps in 10% formalin solution for processing of histopathological studies.

Estimation of biochemical parameters

SGOT and SGPT were estimated by Reitman and Frankel method; ALP was estimated by kind King's method. Total bilirubin and total protein were estimated by Jendrassik and Grofs method and cholesterol oxidase/peroxidases method, respectively.[13-16]

Histopathological studies

The livers from rats were fixed in 10% neutral formalin solution, dehydrated in alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with hematoxylin and eosin for light microscopic analyses.

Statistical analysis

The results are presented as mean ± standard error of mean (n=6 in each group). Analyses were performed using One-way ANOVA followed by Dunnett's multiple for the difference between the control and treatment groups.

RESULT

The preliminary phytochemical studies confirmed the presence of carbohydrates, Phenolic compounds like tannins, flavonoids and saponins.

Table-01: Preliminary Phytochemical screening of the *Tectona grandis* Linn Petroleum ether extract

S.N.	Phytoconstituents	Petroleum ether extract of *Tectona grandis* Linn (PTG)
1.	Carbohydrates	+
2.	Glycosides	+
3.	Saponins	+
4.	Flavonoids	+
5.	Alkaloids	-
6.	Tannins	+
7.	Steroids	+
8.	Amino Acids	-
9.	Proteins	-

Acute oral toxicity study (AOT 425)

Oral administration of petroleum ether extract of *Tectona grandis* up to the dose of 2000 mg/kg to the respective rats did not show any serious adverse effects or mortality observed continuously for 04 hours and everyday for next 14 days. From this data and pilot study performed at laboratory, three different doses 100, 200 and 400 mg/kg were selected for further study.

Hepatoprotective Potential of *Tectona Grandis* Linn in Carbon Tetra Chloride (CCl_4)induced hepatotoxicity

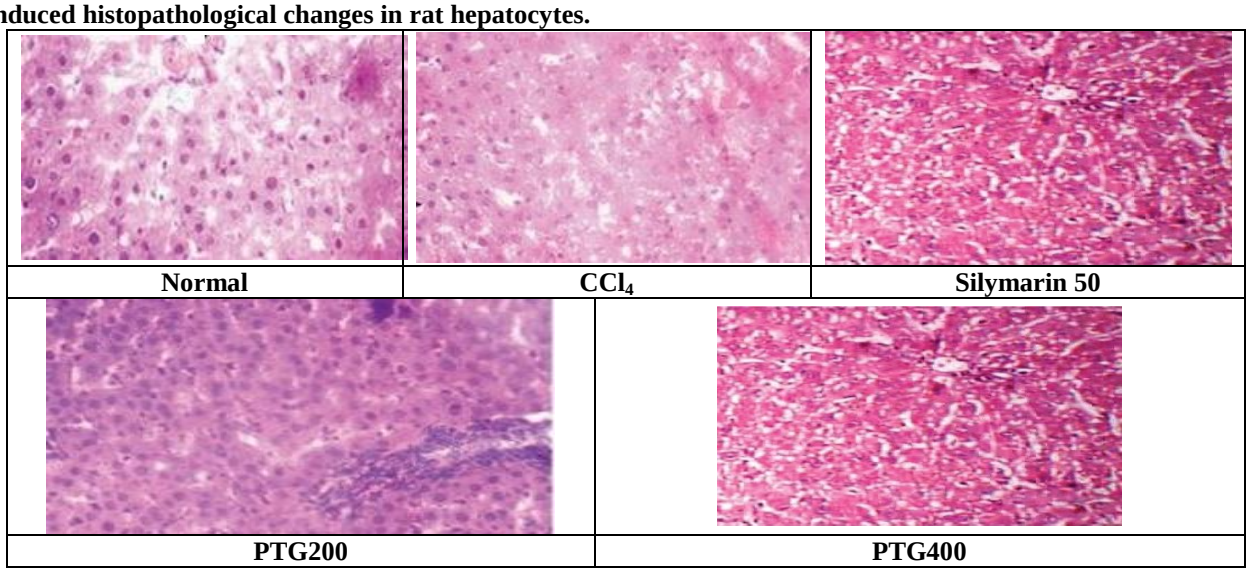
CCl_4 treated group showed significant increase in the levels of AST, ALT and ALP as compared to that of vehicle treated normal control rats. The PTG 400 mg/kg, and Silymarin 50 mg/kg showed significant and equipotent (P<0.01) reduction in elevated levels of these enzymes and showed hepatoprotective effect. PTG 200 mg/kg was effective to reduce AST levels only whereas PTG 100 mg/kg was not significant at all. (Table-02). These biochemical results are further supported with histopathological observations. (Figure-01).

Table-02: Effect of Various drug treatments on different enzyme levels in Carbon Tetra Chloride (CCl_4) induced in rats

Treatment (mg/kg)	AST (U/l)	ALT (U/l)	ALP (mmol/l)
Normal Control (Vehicle-10ml/kg)	127.83 ± 1.16	66.45 ± 2.83	174.59 ± 2.9
CCl_4 Control	242.33 ± 3.16$^{\#\#}$	246.46 ± 2.82$^{\#\#}$	285.06 ± 2.37$^{\#\#}$
PTG-100	238.33± 3.28	241±8.41	281.73 ± 2.64
PTG-200	145.66 ± 2.36**	242.50±4.11	280.89 ± 2.19
PTG-400	134.66 ± 3.31**	151.87±2.16**	230.59 ± 1.64**
Silymarin-50	131.83 ± 1.44**	105.35±5.38**	197.1 ± 1.58**

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- t test*. **P*<0.05, ***P*<0.01.

Figure-01: Effect of Various drug treatments on histopathological changes of Carbon Tetra Chloride (CCl_4) induced histopathological changes in rat hepatocytes.



DISCUSSION

Hepatotoxicity may be defined as the effect of any agent results in a deviation from normal function, morphology and implies chemical/microbial-driven liver damage. It is causes due to drug and its metabolites accumulation or may be due to metabolic inhibition by other drugs or liver damage. Carbon tetrachloride has been extensively studied as a liver toxicant and its metabolites such as trichloromethyl radical(CCl_3-) and trichloromethyl peroxyl radical (CCl_3O_2-) are reported to be involved in the pathogenesis of liver and kidney damage. Liver and kidney are the two important vital organ mostly affected by the drugs and xenobiotics.[17] The massive generation of the free radicals in the CCl_4 induced liver damage significantly reversed by petroleum extract of Tectona Grandis Linn seed (400mg/kg). Histopathalogical profile of liver of control animals Shows normal liver architecture (Fig-01), whereas the Liver section of animals treated with CCl_4 Shows distorted liver architecture with more hepatocytes degenerative changes and necrosis. The Liver section of animals treated with petroleum extract of *Tectona Grandis* Linn Seed shows normal hepatocytes and absence of necrosis. The above reports confirmed the hepatoprotective effect of petroleum extract of *Tectona Grandis* Linn seed in CCl_4 induced hepatotoxicity in rats.

The hepatoprotective role of petroleum extract of *Tectona Grandis* Linn seed might be due to its antioxidant potential mechanism suggesting that the extract of plant may be useful to prevent the CCl_4 induced Hepatotoxicity. More research is required in this View-point to develop a good hepatoprotective drug from seeds of *Tectona Grandis* Linn. Purification of extract and identification of the active principle may yield active hepatoprotective ingredients.

CONCLUSION

The result of the present study clearly indicates that the petroleum extract of *Tectona Grandis* Linn seed possesses potent hepatoprotective activity against CCl_4 induced hepatotoxicity, which claims its traditional use as a hepatoprotective agent.

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