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Hepatoprotective Activity Of Methanolic And Petroleum Ether Extracts Of Tectona Grandis Against Paracetamol Induced Hepatotoxicity

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Abstract: In the present investigation the methanolic and petroleum ether extracts of *Tectona grandis* seeds were evaluated for atiinflammatory activity using paracetamol induced hepatotoxicity model. Three different doses of each extracts i.e. 100, 200 and 400 mg/kg were used in this regard. Results of this study revealed that both the extracts showed significant and dose dependant hepatoprotective activity by normalising the alterations in the hepatic enzyme levels as well as providing protection hepatocellular damage induced by paracetamol which proved the hepatoprotective potential of *Tectona grandis* seeds.
Key Words: *Tectona grandis*, liver, hepatotoxicity, Paracetamol, hepatoprotective.

INTRODUCTION:

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. Such metabolism is always associated with the disturbance of hepatocyte biochemistry and generation of ROS (reactive oxygen species)[1]. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated[2,3]. Liver diseases remain as one of the serious health problems. In spite of phenomenal growth of modern medicine, there are no synthetic drugs available for the treatment of hepatic disorders.

However there are several herbs/herbal formulations claimed have possess beneficial activity in treating hepatic disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India[4].

Literature survey revealed that a wildly grown plant *Tectona grandis* has been traditionally claimed to possess hepatoprotective property but no systematic approach has been made towards documentation of this claim.

Tectona grandis has been scientifically documented to possess many pharmacological activities such as antibacterial, antifungal, antipyretic, antilithiatic, antioxidant, antitumour, wound healing, antiulcerogenic, anti-diabetic, tocolytic, antiangiogenic and hair growth stimulator activities. Some of the major phytoconstitutents reported from *T. grandis* are anthraquinones (tectoquinone; 2-methylanthraquinone), naphthoquinone (lapachol,

juglone), triterpenes (betulin aldehyde), apocarotenoids, gallic acid, rutin, quercitin, ellagic acid derived from various parts of the plant. Others include commonly occurring compounds such as cellulose, holocellulose, lignin and mineral substances[5-10].

Thus the present study was conducted to evaluate the hepatoprotective activity of the of two extracts i.e. methanolic (MTG) and petroleum ether (PTG) extracts of *Tectona grandis* seeds by using paracetamol-induced hepatic injury in rats.

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MATERIALS AND METHODS:

Plant material:

Commercially available dry seeds of *Tectona grandis* Linn. were purchased in the bulk quantity from local market and authenticated by the botanist from the Agriculture College, Pune.

Preparation of extract:

The methanolic and petroleum ether extracts of *Tectona grandis* were prepared using appropriate methods.

Chemicals and drugs:

Paracetamol and silymarin were purchased either from approved vendors or from the local market as applicable.

Preparation of drug solution:

Accurately weighed quantities of both the powdered extracts were dissolved in distilled water to prepare the appropriate stock solution of the drug from which the different doses were administered by selecting the appropriate concentration of the stock solution**.**

Animals:

Wistar albino mice (18 – 22 g) and rats (120 – 150 g) were used. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hr. light 12 hr. dark cycle). All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. (CPSEA/IAEC/PC-02/05-2K11). All the experiments were carried out between 9:00- 16:00 hours and ethical guidelines were strictly followed during all the experiments. The respective animals were shifted from animal house to the laboratory 1 hour prior to the start of the experiment to avoid any error in the results due to change in location and environment.

Preliminary Phytochemical Analysis of extracts:

The both extracts were tested for the presence of various chemical constituents in it (Khandelwal, 2006; Kokate, 1997)[11].

Preliminary acute toxicity test:

Healthy adult male wistar albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (AOT 423) suggested by the OECD-2000 (OECD Guidelines). The rats were continuously monitored immediately from dosing up to next 4 hours for behavioral, neurological and autonomic profiles. The animals were observed after 24 hours and everyday for next 14 days for any sign of toxicity or mortality[11].

Evaluation of hepatoprotective activity using paracetamol model[12]

Rats were divided into nine groups, each group containing six rats. Group I (normal control) received distilled water (10 ml/kg; daily p.o.). Group II (induction control) received paracetamol 3 gm/kg p.o. Groups III–VIII received MTG (100, 200 and 400 mg/kg, p.o.) and PTG (100, 200 and 400 mg/kg, p.o.) respectively, for 10 days and single dose of paracetamol 3 gm/kg p.o. on 11th day. Group IX received Silymarin (50 mg/kg, p.o.) for 10 days and single dose of paracetamol 3 gm/kg p.o. on 11th day. The duration of treatment was decided on pilot study. 24 hours after paracetamol administration serum parameters aspartate amino transferase (AST), serum alanine amino transferase (ALT), serum alkaline phosphatase (SALP) were estimated to assess hepatoprotective. Thereafter, all the rats were sacrificed and histopathological examination of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of Kupffer cells and lymphocytes was carried out (Setty *et al.*, 2007).

RESULTS:

Preliminary Phytochemical screening of the *Tectona grandis* Linn extract

Preliminary phytochemical analysis of methanolic extract of *Tectona grandis* showed the presence of glycosides, flavonoids, tannins, steroids while petroleum ether extract showed the presence of carbohydrates, saponins, glycosides, flavonoids, tannins, steroids.

Acute oral toxicity study (AOT 425)

Oral administration of methanolic and 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.

Evaluation of hepatoprotective activity using paracetamol model

Paracetamol administration exhibited significant increase in the levels of AST, ALT and SALP as compared to vehicle treated normal control and thereby indicated hepatotoxicity.

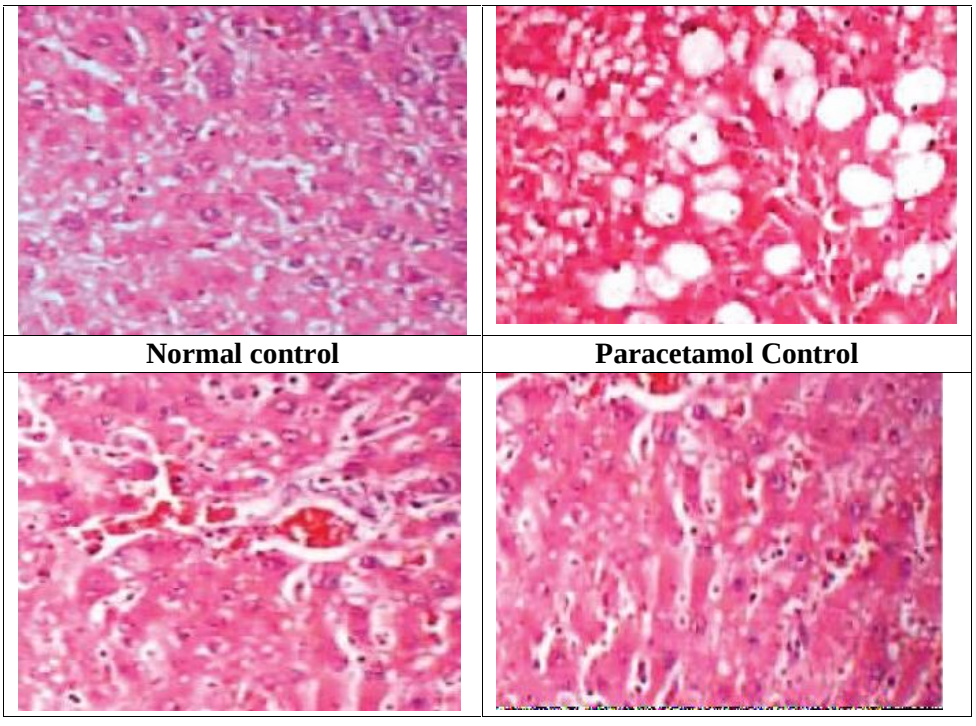
The pretreatment with MTG 200, 400 mg/kg, PTG 400 mg/kg and Silymarin 50 mg/kg showed significant and equipotent reduction in these levels when compared against paracetamol treated group indicating their hepatoprotective potential. PTG 200 mg/kg was less significant ($P<0.05$) towards SALP than AST and ALT ($P<0.01$) whereas as MTG 100 and PTG 100 were not significant in this regard (Table 1). These biochemical observations further supported by histopathological outcome (Figure 1).

Table 1. Effect of Various drug treatments on different enzyme levels in Paracetamol induced hepatotoxicity in rats.

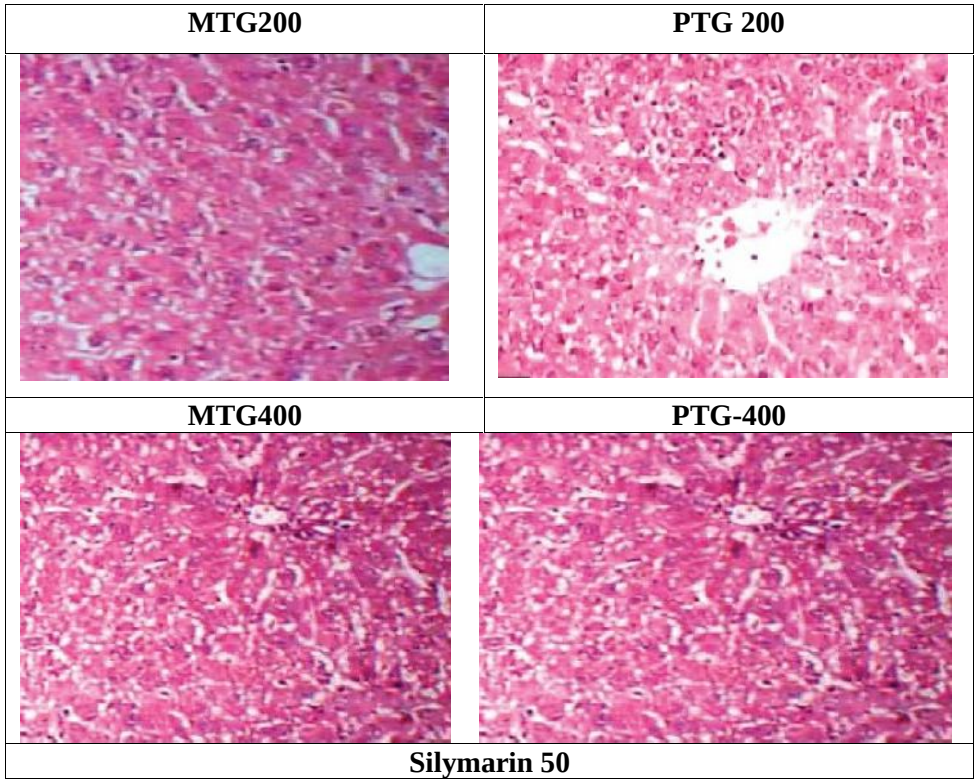
Treatment (mg/kg)	AST(U/l)	ALT (U/l)	SALP (mmol/l)
Normal Control (Vehicle-10ml/kg)	127.83±1.16	90 .2±0.58	174.59 ± 2.90
Paracetamol-30	237.5±2.68$^{\#\#}$	201.97+± 2.8$^{\#\#}$	276.72 ± 2.91$^{\#\#}$
MTG-100	229.00 ± 3.54	197.33±3.74	279.16 ± 1.89
MTG-200	142.50±1.89**	101.82±1.8**	250.09 ± 4.28**
MTG-400	133.33±1.68**	99.41±2.61**	207.66 ± 2.88**
PTG-100	236.33 ± 2.74	201±3.77	281.73 ± 2.64
PTG-200	201.00±3.94**	147.00±4.15**	261.56 ± 2.09*
PTG-400	159.16±2.84**	102.83±2.35**	223.92 ± 2.24**
Silymarin 50	131.83±1.44**	105.35±**	188.76 ± 4.44**

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 1: Effect of Various drug treatments on Paracetamol induced histopathological changes in rat hepatocytes.



Normal control | **Paracetamol Control**



DISCUSSION:

Attempts are being made to develop new drugs from traditional medicines for different liver diseases and toxicities[13]. In the present study, we have attempted to establish the hepatoprotective effect of *Tectona grandis*, a traditional drug in experimental liver damage by evaluating the methanolic and petroleum ether extracts of *Tectona grandis* against paracetamol induced hepatotoxicity in rats.

Paracetamol (acetaminophen) is a commonly and widely used analgesic and antipyretic agent. Hepatotoxic doses of acetaminophen deplete the normal levels of hepatic glutathione, when NAPQI covalently binds to cysteine groups on proteins to form 3-(cystein-S-yl) acetaminophen

Adducts[14]. The glutathione protects hepatocytes by combining with the reactive metabolite of

paracetamol thus preventing their covalent binding to liver proteins[15]. In living systems, liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities such as ALT, AST, SALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage[16]. In the present investigation it was observed that the animals treated with paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels are said to be the indicators of hepatic structural integrity. The normalization of serum markers by MTG and PTG at higher suggests that they are able to condition the hepatocytes so as to protect the membrane integrity against paracetamol induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells[14-16]. Serum ALP and bilirubin levels, on the other hand are related to hepatic cell damage. Increase in serum level of ALP is due to increased synthesis in presence of increasing billiary pressure. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell[17,18].

The histopathological observations in paracetamol-treated rats showed severe necrosis, with disappearnce of nuclei. This could be due to the formation of highly reactive radicals because of oxidative threat caused by paracetamol. All these changes were very much reduced histopathologically in rats treated with higher doses of MTG and PTG. Based on the above results, it could be concluded that both methanolic as well as petroleum ether extracts of *Tectona grandis* exert significant hepatoprotection against paracetamol-induced toxicity.

CONCLUSION:

In summary, the present study provides evidence that both the methanolic as well as petroleum ether extracts of *Tectona grandis* are possess significant

hepatoprotective potential. The exact role of individual phytoconstituents needs to be illustrated using suitable bio-analytical techniques to extrapolate exact mechanism of this action.

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