ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Study on Hepatoprotective Activity of Methanolic Extracts of Tectona Grandis Seeds.

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ABSTRACT

In the present investigation the Methanolic extracts of *Tectona grandis* seeds were evaluated for hepatoprotective activity using CCl_4 and Ranitidine induced hepatotoxicity model. Three different doses of each extracts i.e.100, 200 and 400mg/kg were used in this regard. Results of this study revealed that both the extracts showed significant and dose dependant hepatoprotective activity by normalizing the alterations in the hepatic enzyme levels as well as providing protection hepatocellular damage induced by CCl_4 and Ranitidine which proved the hepatoprotective potential of *Tectona grandis* seeds.

Keywords: Tectona Grandis, liver, CCl_4, Ranitidine, hepatoprotective,

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ISSN: 0975-8585

INTRODUCTION

Liver plays an important role in regulation of physiological processes, involved in several vital functions such as metabolism, secretion and storage. Liver also detoxifies variety of drugs and xenobiotics and secretes bile that has an important role in digestion. These are among the most serious ailments classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (fibrosis of liver). Liver diseases are mainly caused by toxic chemical drugs eg. ccl4,Paracetamol, anti-tubercular, anticancer agents, ferrous sulphate or alcohol, some natural toxins such as peptides of Amanita phalloides, pyrrolizidines, and the toxin of cycad nut. Most of the hepatotoxic chemicals damage liver cells by inducing lipid peroxidation and other oxidative cell damage. It has been estimated that about 90% of acute hepatitis is due to viruses and major viral agents involved are hepatitis A, B, C, D, E and G. Among these, hepatitis B infection often results in chronic liver diseases and cirrhosis of liver.

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acuteliver failures [1].

AYURVEDA, an ancient Indian system of healing, described various plants for the treatment of hepatotoxicity. Since times plants have been used therapeutically in variety of conditions and with the advent of modern synthetic drugs and their convenience of standardized dosage forms, dramatic efficacy in acute conditions and most of all simplicity of usage, there was a decline in the use of plant medicines till the herbal revolution. In contrast to the narrow spectrum of activity of synthetic drugs with their attendant risk of side effects, herbal drugs as traditionally used are often mild in action and need to be taken for a long period to be effective especially in chronic conditions.

In spite of the tremendous scientific advancement in the field of hepatology in recent years, liver problems have been on the rise. Many plant based drugs are found to be hepatoprotective in nature like Andrographis paniculata, Boerrhavia diffusa, Calotropis procera, Fumaria indica, Garcinia cambogia, Luffa acutangula, Mamordic subangulata, Naragamia alata, Nigella sativa and Trigonella Foenum graecum. Jaundice and hepatitis are two major liver disorders, with a high toll of death rate. At present only few hepatoprotective allopathic drugs are available for the treatment of liver disorders. Hence people are adapting different extracts from the plants for the treatment of liver disorders. Thus i n the present study was conducted to evaluate the hepatoprotective activity of methanolic extracts of *Tectona grandis(MTG)* seeds by using CCl_4 and Ranitidine induced hepatic injury in rats.

MATERIALS AND METHODS

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.*Tectona grandis* Linn seeds were subjected to size reduction to a fine powder with the help of mixer grinder.The extracts are prepared as follows

Preparation of alcoholic extract [2]:

The powder was packed in a soxhlet apparatus and extracted with 95% methanol for 18 hrs. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get Methonolic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated.

Preliminary phytochemical investigations:

The preliminary phytochemical investigations were carried out with methonolic 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 [2-4]. All the chemicals and reagents used were of analytical grade. The results are compiled in Table-01

Determination of acute toxicity (LD50) [5, 6]:

The acute toxicity of methonolic 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 ED50.

Determination of hepatoprotective activity:

Evaluation of hepatoprotective activity using Carbon tetrachloride (CCl4) model [7]:

Albino rats weighing between 150-200gm each group containing six animals will be divided in six groups.

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 - Methonolic extracts of seeds of *Tectona Grandis*(100 mg/kg, p.o)
Group E - Methonolic extracts of seeds of *Tectona Grandis*(200 mg/kg, p.o)
Group F - Methonolic extracts of seeds of *Tectona Grandis*(400 mg/kg, p.o)

Experimental procedure:

Group I (normal control) received distilled water (10 ml/kg) for 5 days. Group II (induction control) received distilled water (10 ml/kg) for 5 days and CCl_4 3 ml/kg, s.c., 1:1 dilution with olive oil on 3rd day. Groups III received Silymarin (50 mg/kg, p.o.) for 5 days and Group IV –VI received MTG (100, 200 and 400 mg/kg) and CCl_4 (3 ml/kg) induction on 3rd day. On the 6th day, all rats were sacrificed under ether anesthesia, and blood and liver samples were collected. The blood was allowed to clot for 30 minutes; serum was separated by centrifuging at 37^{0}C and was used for biochemical estimations. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formosaline.

Ranitidine induced hepatotoxicity [8-10]:

Albino rats weighing between 150-200gm each group containing six animals will be divided in six groups.

Group A - Normal Control (vehicle treated, p.o)
Group B - Toxicant (Ranitidine 50mg/kg, i/m)
Group C - Standard (Silymarin 50mg/kg, p.o)
Group D - Methonolic extracts of seeds of *Tectona Grandis*(100 mg/kg, p.o)
Group E - Methonolic extracts of seeds of *Tectona Grandis*(200 mg/kg, p.o)
Group F - Methonolic extracts of seeds of *Tectona Grandis*(400 mg/kg, p.o)

Experimental procedure:

Wistar rats weighing between 150-200g was divided into nine groups of six rats in each. Group A were administered vehicle for 21 days and served as normal control, group B with ranitidine (50mg/kg, i.m), and group C with silymarin (100mg/kg, p.o) which was serve as standard. Animals in group D, E, F were treated with three different doses (low, medium and high) of methonolic extracts of seeds of *Tectona Grandis.* For 21 days, animals of group B, C, D, E, and F were intoxicated with ranitidine (50mg/kg, i/m). On the 22nd day, after recording thiopentone sleeping time in all animals of all groups was anesthetized with ether. Blood was collected from retro orbital puncture, later sacrificed by overdose of ether. Livers was removed, washed with saline, weighed and stored in 10% formalin for histological studies.

Assessment of liver functions:

Blood samples of the rats were withdrawn from retino- bulbar venous plexus with the help of a glass capillary under light anesthesia and were kept at room temperature for 2 hours, so that the coagulation process was completed. The blood samples were centrifuged and serum thus separated was used for the estimation of AST, ALT, and SALP. Animals were then sacrificed and dissected. Their livers were taken out, washed with water, dried gently with filter paper and preserved in 10% formolsaline. The levels of AST, ALT and SALP were measured.

Histopathological investigations:

The liver samples fixed for 48 hours in 10% formolsaline were dehydrated by passing successively in different mixtures of ethyl alcohol–water (50, 80, and 95%, and finally in absolute alcohol), cleared in xylene and embedded in paraffin. Sections (4–5mm thick) were prepared and then stained with hematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of Kupffer cells and lymphocytes.

RESULTS

Table 1: Preliminary Phytochemical screening of the *Tectona grandis* Linn extract

S.N.	Phytoconstituents	Methanolic extract of *Tectona grandis* Linn (MTG)
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 methanolic 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* Linnin Carbon Tetra Chloride (CCl_4)induced hepatotoxicity:

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 MTG 200, 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. Whereas MTG 100 mg/kg was not significant at all (Table-02). These biochemical results are further supported with histopathological observations. (Figure-01).

Hepatoprotective Potential of *Tectona Grandis* Linnin Ranitidineinduced hepatotoxicity

Ranitidine treated group showed significant increase in the levels of AST, ALT and ALP as compared to that of vehicle treated normal control rats. The MTG 100, MTG 200, 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 (Table-03). These biochemical results are further supported with histopathological observations (Figure-02).

ISSN: 0975-8585

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.

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

CCl4 is commonly used for free radical induced liver injury. [11-14] CCl4 toxicity results from bioactivation of CCl4 into trichloromethyl free radical by cytochrome P450 system in liver microsomes and consequently causes lipid peroxidation of membranes that leads to liver toxicity. [12, 15] Inthe presentinvestigation it was observed that the animals treated with CCl4 elicited the elevation in the levels of liver marker enzymes (AST, ALT, and ALP) resulting in a significant hepatic damage. Whereas pretreated with different doses of Methonolic extracts of seeds of *Tectona Grandis* (200, 400 mg/kg)liver marker enzymes significantly decreases. It indicates Methonolic extracts of seeds of *Tectona Grandis* shows protection against CCl4 induced hepatotoxicity.

Liver injury induced by ranitidine is due to its metabolite, which may leads to hepatic oxidative damage and one of its metabolite generates immunoallergic reaction. [16] It also produces a reaction as reflected by infiltration of hepatocytes with ranitidine 50 or 30 mg/kg. Severe inflammatory changes with collagenous septa beginning to form after pronounced centrilobular and bridging necrosis. In the parenchyma there was focal liver cell necrosis with some accumulation of histocytic elements and slight steatosis and cholestasis. Portal tract shows fibrosis, bile duct proliferation, and infiltrate consisting of lymphocytes, plasma cells, polymorphs, and eosinophils. Liver injury is manifested in terms of increased levels of serum aminotransferases, modest hepatic infiltration by both lymphocytes and eosinophils and slight focal hepatocellular necrosis also causes liver cholestasis associated with increased plasma bilirubin and alkaline phosphatase levels. [8-10] In Ranitidine induced hepatotoxicity model different doses of Methonolic extracts of seeds of *Tectona Grandis (100, 200, 400 mg/kg)* liver marker enzymes significantly decreases. It indicates Methonolic extracts of seeds of *Tectona Grandis* also shows protection against ranitidine induced hepatotoxicity.

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

Table 2: 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$^{\#\#}$
Silymarin-50	131.83 ± 1.44**	105.35±5.38**	197.1 ± 1.58**

MTG-100	232.83 ± 2.56	237.22±5.61	270.83 ± 3.49*
MTG-200	139.5 ± 2.66**	168.5±3.4**	241.75 ± 3.79**
MTG-400	134.5 ± 1.11**	147±3.45**	217.16 ± 3.17**

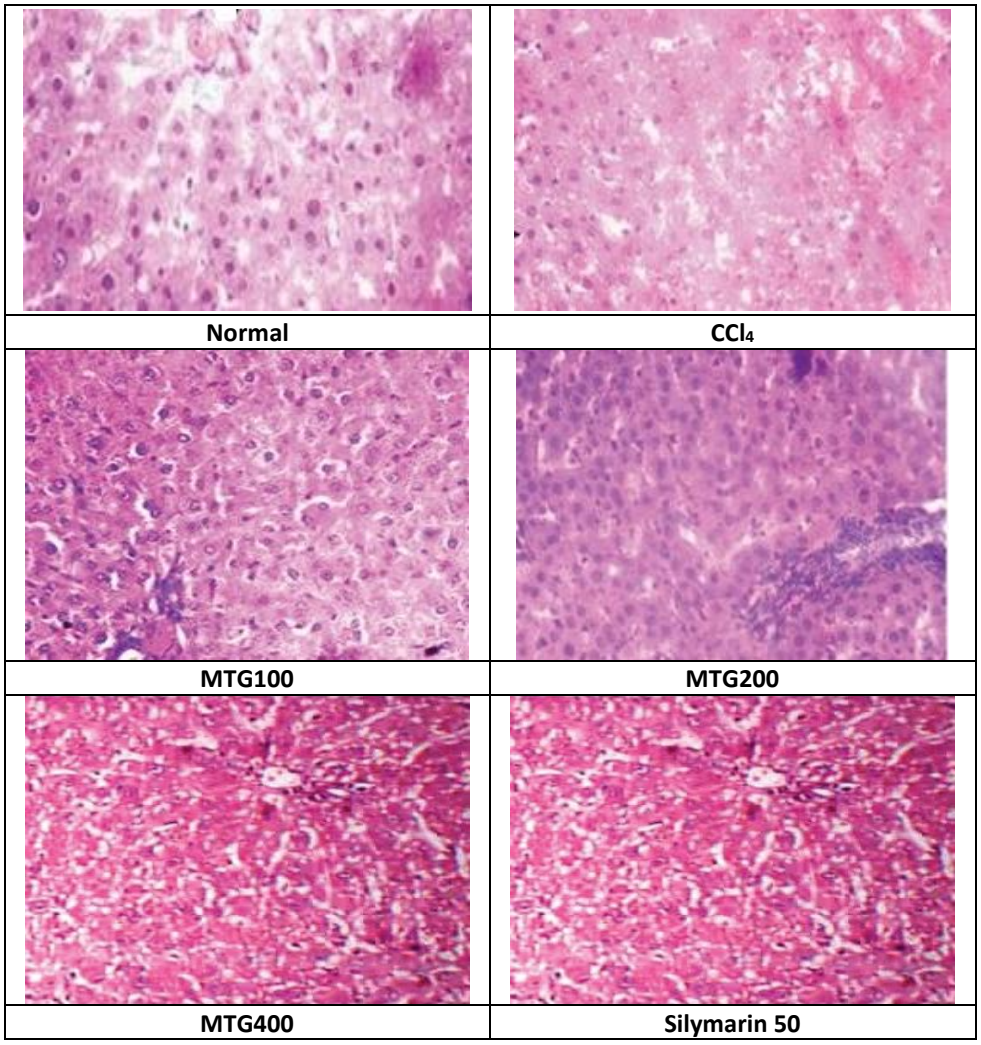
Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- t test*. *P<0.05, **P<0.01

Table 3: Effect of Various drug treatments on different enzyme levels in Carbon Ranitidine induced in rats

Treatment (mg/kg)	AST (U/l)	ALT (U/l)	ALP (mmol/l)
Normal Control (Vehicle-10ml/kg)	107.23 ± 15.35	40.280 ± 4.11	107.22 ± 7.39
RanitidineControl	282.22 ± 13.15	137.48 ± 3.69	251.89 ± 6.50
Silymarin-50	108.25** ± 2.99	44.53** ± 3.40	115.51** ± 5.62
MTG-100	267.31^{ns}± 9.77	118.27** ± 2.23	222.34* ± 7.84
MTG-200	176.82** ± 4.37	95.267** ± 4.53	173.02**± 1.90
MTG-400	159.17** ± 2.63	72.392** ± 2.38	136.92** ± 5.58

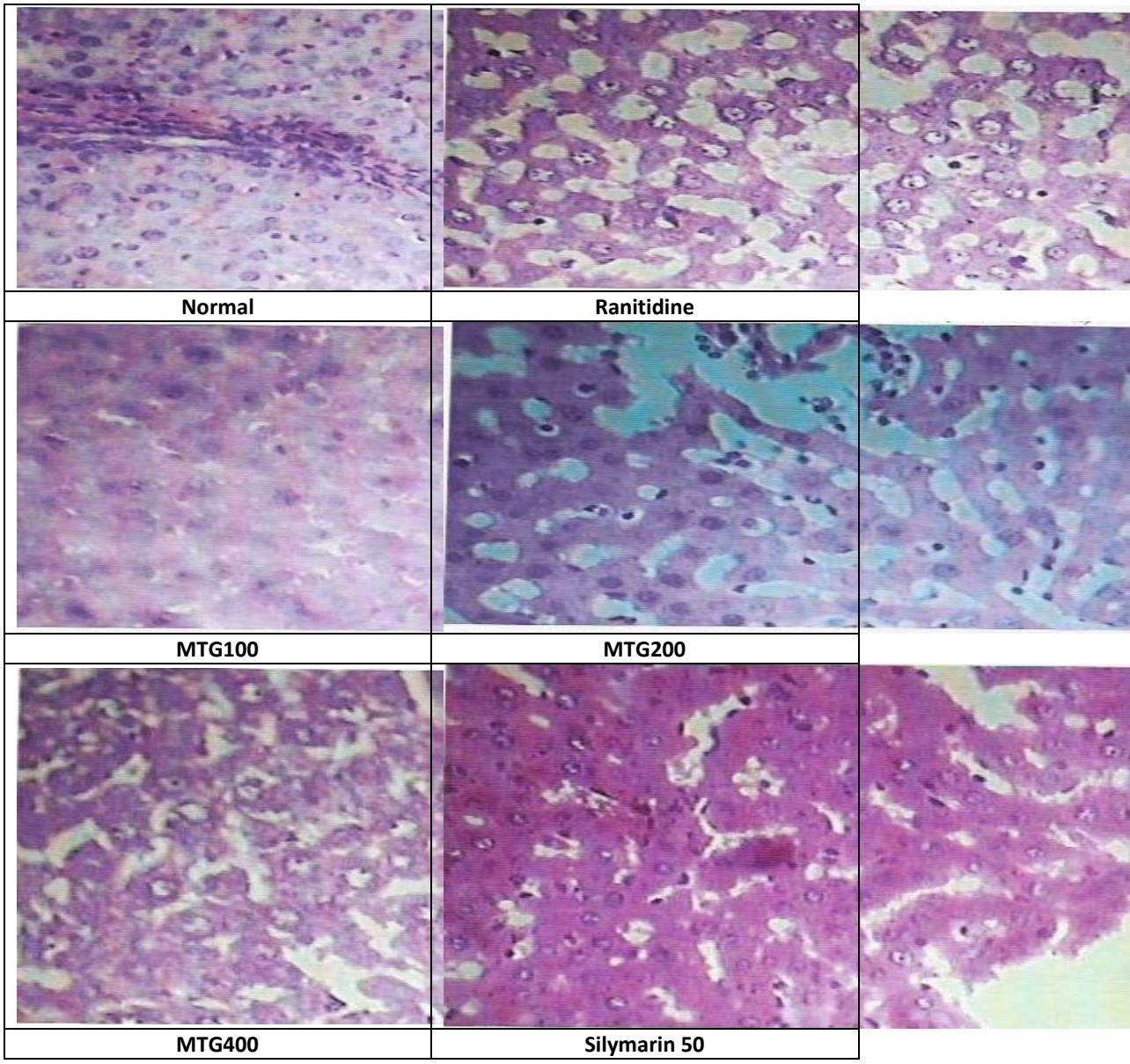
Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- t test*. *P<0.05, **P<0.01

Figure 1: Effect of Various drug treatments on histopathological changes of Carbon Tetra Chloride (CCl_4) induced histopathological changes in rat hepatocytes.



ISSN: 0975-8585

Figure 2: Effect of Various drug treatments on histopathological changes of Ranitidine induced histopathological changes in rat hepatocytes.



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