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# Evaluation of Anti-fibrotic effect of *Lagerstroemia Speciosa (L) pers.* on Carbon Tetrachloride Induced Liver Fibrosis

**V. Vinoth Prabhu*, N. Chidambaranathan, G. Nalini, S. Venkataraman, S. Jayaprakash, M. Nagarajan**
K.M. College of Pharmacy, Madurai-625 107, Tamil Nadu, India

## Abstract
This study was carried out to investigate the effect of alcoholic extract of *Lagerstroemia speciosa (L) pers.* against carbon tetrachloride ($CCl_4$) induced liver fibrosis in male albino Wistar rats (150-200gm). Liver fibrosis was induced by twice/week administration of $CCl_4$ at a dose of 1ml/kg body weight, mixed with an equal volume of corn oil. The extent of liver fibrosis was assessed by the content of hydroxyproline in liver, serum level of Asparate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Billirubin and by histological studies. Treatment with alcoholic extract of *Lagerstroemia speciosa (L) pers.* (100 mg/kg body weight) orally, reduced the hydroxyproline content of the liver, various serum enzymes level and total billirubin. The architecture of liver deranged by $CCl_4$ showed improvement following administration of the extract. These observations confirm the potent antifibrotic effect of the extract.

## Keywords
Lagerstroemia speciosa, Banaba, Hydroxyproline, Aspartate transaminase, Alanine transaminase, Alkaline phosphatase.

## Introduction
Fibrosis is seen as a scar formation in liver as the liver tries to repair the damaged tissue[1].Hepatic fibrosis is the result of chronic viral, toxic, autoimmune or cholestatic liver injury[2]. Viral infection seems to be a crucial factor in liver fibrosis. Majority of persons with chronic Hepatitis C virus (HCV) infection develop liver fibrosis[3] that progresses to cirrhosis within 20 years in an estimated 20-30% patients[4]. Also it was observed that Human Immuno Deficiency virus (HIV) co-infection in HCV infected patients accelerate the progression of fibrosis[5]. Likewise, numerous chemicals and drugs can harm the liver[6]. In many experimental fibrotic models, $CCl_4$ has been used to induce hepatic injury[7, 8]. Some workers have used N-nitro dimethyl amine (NNDA) to injure rat liver[9]. Likewise bile duct ligation technique has been used by many workers to induce fibrosis[10-12]. In this study $CCl_4$ induced liver fibrosis was used as a model to evaluate the antifibrotic effect of *Lagerstroemia speciosa (L) pers.*

***Corresponding Author:**
vvinothprabhu@yahoo.com

It is a small tree that can grow as tall as 20 m also called as Banaba belonging to the family Lythraceae is a medicinal big plant that grows in India, Southeast Asia, and Philippines. This small tree has been investigated by very few workers and reported for hypoglycemic effect[13]. Recently it has been shown that *L. speciosa* leaves contain corosolic acid, as a active ingredient, which exhibits antioxidant and antidiabetic activity as like as vitamin E, which can protect cell membranes from lipid peroxidation by scavenging free radicals[14]. As $CCl_4$ induces liver fibrosis by getting converted to free radical which damages the liver, through lipid peroxidation and as the plant is reported to inhibit quartz induced lipid peroxidation thereby *L. speciosa* leaves was chosen to evaluate its potential against $CCl_4$ induced liver fibrosis.

## Materials and Methods
### Plant Material
The Fresh leaves of *Lagerstroemia speciosa (L) pers.* were collected from wild area near to Mannargudi, Tiruvarur District, Tamil Nadu, India and the same were authenticated by Prof. Dr. G.S.V. Murthy -Botanical Survey of India (BSI), Coimbatore and a voucher specimen No:

BSI/SRC/5/23/09-10/Tech-682 is allotted for *Lagerstroemia speciosa (L) pers.* (Fig 1) and the herbarium was deposited in our University laboratory for further reference.

**Fig. 1. Leaves of *Lagerstroemia speciosa (L) pers.***

**Animals**

Healthy Male albino Wistar rats weighing between (150-200 g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai. They were housed in poly propylene cages (4 per cage), maintained in standard lab conditions at 25 ± 2$^{o}$C under a 12-h light/dark cycle. They were fed with standard pellet diet and water *ad libitum*. This study was approved by the Institutional Animal Ethical Committee (IAEC) and the all the animal experiments were performed according to the strict guidelines prescribed by CPCSEA.

**Preparation of Ethanolic Extract**

The leaves were shade dried and made into small pieces. These pieces were again shade dried and pulverized to the course powder and passed through sieve No. 20 and was retained on sieve No. 40. The powdered materials (leaf) was extracted with ethanol in the ratio 1:5 by continuous hot percolation by placed in a thimble of the Soxhlet extraction apparatus attached to the mouth of a round bottomed flask. Some boiling chips were added into the flask to avoid bumping during heating. The extraction of leaf was completed in 48 hours. The residue (yield: 30% w/w) was subject to Phytochemical screening.

**Phytochemical Screening**

Phytochemical screening of ethanolic extract residue of *Lagerstroemia speciosa (L) pers.* were carried out using conventional protocol for detecting the presence of different phytochemical active constitutes in the plant respectively[15] and the result is shown in (Tab.1).

**Induction of Liver Fibrosis by Carbon Tetrachloride**

$CCl_4$ was given to rats orally twice a week for 28 days at the dose of 1 ml/kg body weight mixed with an equal volume of corn oil [16]. Three days after the last dose, rats were sacrificed under light ether anesthesia and blood and liver samples were collected for biochemical and histopathological studies respectively.

**Treatment with the Ethanolic Extract of *Lagerstroemia speciosa (L) pers.***

The ethanolic extract was diluted with distilled water and given orally by gavage for 28 days at the daily dose of 100 mg/kg body weight; the control group received equal amount of distilled water, given orally for 28 days. For comparison, a group of normal rats and another group treated with extract alone were used. Throughout the study the body weight of the animals was recorded every day (Table 2).

**Estimation of Serum Biochemical Parameters**

After 28 days the rats were sacrificed under light ether anesthesia and blood was collected by cardiac puncture. A part of it was used for biochemical estimation and centrifuged at 3000 rpm to obtain serum. The levels of Aspartate Transaminase (AST), Alanine Transaminase (ALT) [17], Alkaline Phosphatase (ALP) [18] and Total Billirubin were estimated by standard procedures (Table 1) and the platelet count was done.

**Determination of Hydroxyproline Content in Liver**

The hydroxyproline content of liver was determined by the method[19] and the specimens of liver were weighed and hydrolyzed completely in 6M HCL, a fraction of the sample was derivatised using Chloramines T solution and Erhlich's reagent. The optical density was measured at 558nm. The results are shown in (Table 2).

**Histopathological Studies**

Liver samples were weighed and fixed rapidly with 10% neutralized formalin (Ph 7.4). Sections of liver fixed in paraffin were prepared and stained with hematoxylin and eosin and observed for pathological changes.

**Statistical Analysis**

The values are expressed as mean ± SEM. The analysis of data was done with one-way ANOVA followed by Newman Keul's multiple range tests. Differences below $P<0.05$ implied significance.

**Table 1:** Preliminary Phytochemical Screening of *Lagerstroemia speciosa (L) pers.*

| S.No | Phytochemical Test | Test Methods/Reagents | Results |
|---|---|---|---|
| **1** | Alkaloids | Mayer's<br>Dragendroff's | +<br>+ |
| **2** | Carbohydrates & Glycosides | Molisch's<br>Borntrager's | -<br>- |
| **3** | Cardiac glycosides | Legal's test<br>Kellerkilliani test | -<br>- |
| **4** | Sugars | Fehling's test<br>Benedict's test | -<br>- |
| **5** | Steroids | Liebermann's Burchard test<br>Salkowski test | -<br>- |
| **6** | Tannins | Lead solution<br>Aqueous gelatin solution | ++<br>++ |
| **7** | Proteins | Million's test<br>Biuret test<br>Ninhydrin test<br>Xanthoprotein test | -<br>-<br>-<br>- |
| **8** | Terpenoids | Noller's test | ++ |
| **9** | Flavonoids | Shinoda test | - |
| **10** | Anthocyanins | NaoH and Conc. Sulphuric acid | - |
| **11** | Quinones | NaoH test | - |

+ indicates the presence of phytochemical, ++ Indicates the presence of more quantity,
- indicates the absence of phytochemical.

## Results

### Serum Parameters

Treatment with $CCl_4$ altered various tissue and serum parameters and the architecture of liver. In $CC1_4$ rats the serum parameters AST, ALT, and ALP were significantly elevated. The billirubin level was also high. However in $CCl_4$ + extract treated rats the serum level of these enzymes and billirubin was significantly low when compared to $CCl_4$ alone treated rats. The platelet count was also low in $CCl_4$ treated rats. But following treatment with the extract the platelet count increased significantly. The results are given in (Table 2).

### Tissue Parameters

The main tissue parameters assessed were hydroxyproline content and weight of liver. Hydroxyproline was elevated following $CCl_4$ treatment. Treatment with the extract reduced this parameter. Liver weight was increased in $CCl_4$ treated rats which was reduced by the extract. The results are given in (Table 3).

### Histopathological Changes

The architecture of liver was completely intact in normal rats. In $CCl_4$ treated rats the liver specimens showed parenchyma with sheets of hepatocytes showing hydropic and fatty changes and areas of mild necrosis. These pathological changes were much reduced in liver specimens of rats treated with extract. The rats treated with the extract alone did not show deviation in the architecture of liver. The result of histopathological studies is given in (Fig. 1 to 3).The group treated with the extract alone was found to be normal in respect of serum, tissue parameters and histopathology.

## Discussion

Liver, the largest internal organ is composed of 6 major cell types' viz. hepatocytes, bile duct epithelial cells, kupffer cells, hepatic stellate cells, sinusoidal epithelium and pit cells [20]. Large literature supports that among their cells, hepatocytes and bile duct epithelial cells, and hepatic stellate cells are involved in cellular necrosis and fibrosis.

**Table 2:** Effect of *Lagerstroemia speciosa (L) pers.* extract on the biochemical parameters of rats treated with $CCl_4$

| Groups | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | Total Billirubin (mg/dl) | Platelet count (in lakhs/cc) |
|---|---|---|---|---|---|
| Normal Rats | 81.6 ± 2.0 | 36.2 ± 2.2 | 577.3 ± 6.8 | 0.3 ± 0.04 | 3.5 ± 0.02 |
| Normal Rats Treated With *L.S.* Extract | 96.2 ± 7.2 | 27.3 ± 3.2 | 559.9 ± 13.8 | 0.87 ± 0.02 | 3.2 ± 0.08 |
| $CCL_4$ Treated Rats | 665.3 ± 13.3 | 595.3 ± 3.6 * | 1124.1 ± 13.8* | 2.34 ± 0.12* | 1.4 ± 0.12 * |
| $CCL_4$ + *L.S.* Extract Treated Rats | 321.8 ± 15.8 *[a] | 391.83 ± 9.9*[a] | 819.2 ± 13.2 *[a] | 1.64 ± 0.03 *[a] | 2.7 ± 0.07*[a] |

Data are mean ± SEM. n=6, Newman Keul's multiple test was used (P<0.05)
***** Significantly different from normal rats
***[a]** significantly different from $CCl_4$ treated rats.

**Table 2**: Hydroxyproline content of liver, body weight and liver weight following various treatments

| Treatment | Hydroxyproline [Content (µg(g liver)$^{-1}$)] | Body Weight | | Liver Weight (g) | Liver Weight/ Bodyweight |
|---|---|---|---|---|---|
| | | Day 0 | Day 28 | | |
| Normal Rats | 220 ± 11.7 | 160.1 ± 4.1 | 4.4 ± 0.28 | 4.4 ± 0.28 | 0.027 ± 0.01 |
| Normal Rats Treated With *L.S.* Extract | 200 ± 5.8 | 163.3 ± 3.3 | 4.2 ± 1.3 | 4.2 ± 1.3 | 0.026 ± 0.03 |
| $CCL_4$ Treated Rats | 601 ± 17.5* | 168.16 ± 1.5 | 15.1 ± 2.3* | 15.1 ± 2.3* | 0.096 ± 0.02 |
| $CCL_4$ + *L.S.* Extract Treated Rats | 450 ± 6.1*[a] | 164.6 ± 4.5 | 5.2 ± 1.9*[a] | 5.2 ± 1.9*[a] | 0.05 ± 0.0 |

Data are mean ± SEM. n=6, Newman Keul's multiple test was used (P<0.05)
***** Significantly different from normal rats
***[a]** Significantly different from $CCl_4$ treated rats.

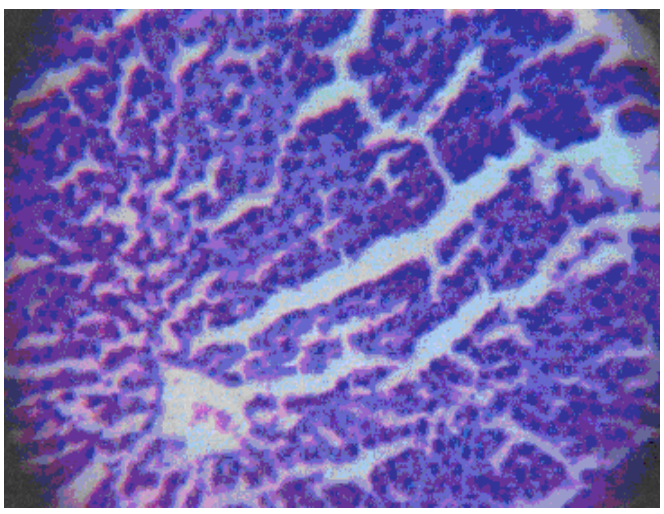

**Fig. 2:** Liver tissue sections from a normal rat showing normal lobular architecture and cell structure

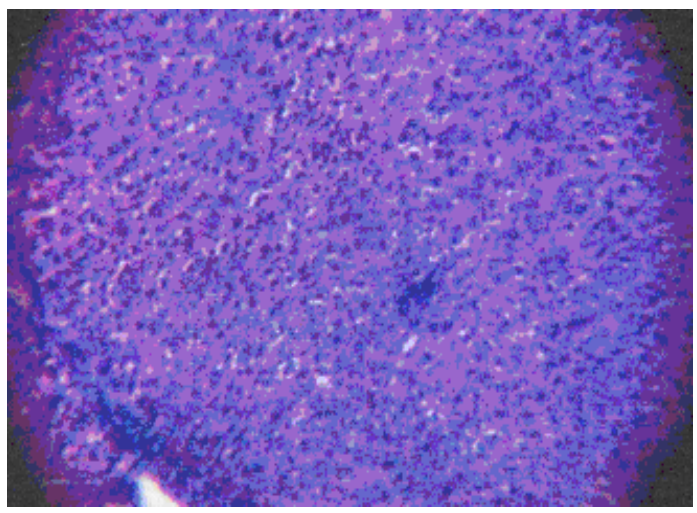

**Fig. 3:** Liver tissue sections of a rat receiving only CCl4 showing extensive hepatocellular damage

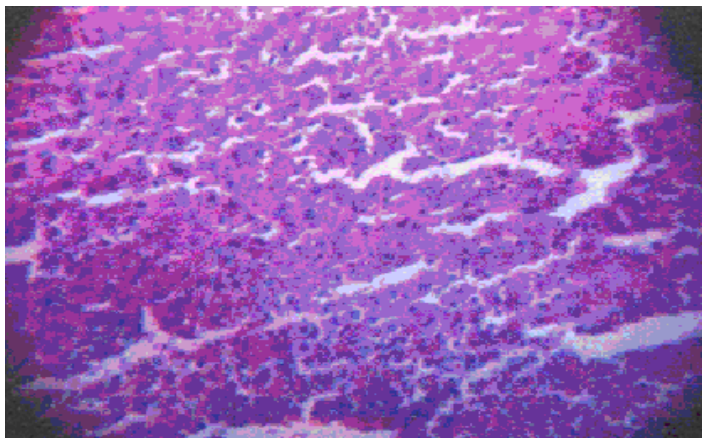

**Fig. 4:** Liver tissue sections of a rat with CCl4 and 100mg/kg *Lagerstroemia speciosa (L) pers.* showing minimal hepatocellular necrosis

The hepatocytes and bile duct epithelial cells when chronically damaged and if fail to recover from chronic damage, undergo either cellular necrosis and apoptosis [21] or fibrosis due to synthesis and secretion of collagen from hepatic stellate cells [22, 23]. Hepatic stellate cells normally act as stores of Vitamin A. However when injured repeatedly, they get activated. There is overwhelming evidence that activated hepatic stellate cells are producers of fibrotic neomatrix. Such activated cells lose their Vitamin A content and start producing hepatic extra cellular matrix including collagens type I and type II which are fibrillar collagens [24]. Thus collagen content in the extra cellular matrix is high in fibrosis, the extent of which could be assessed by the hydroxyproline content[25]. Many earlier studies have reported high hydroxyproline content in association with liver fibrosis. Our results also showed a high hydroxyproline content in $CCl_4$ treated rats indicating the progression of fibrosis, which was brought down by co-treatment with the extract. In our study, $CCl_4$ is the toxicant that was used to damage liver. $CCl_4$ is converted to trichloromethyl radical by the enzyme cytochrome $P450_s$, which initiates lipid per oxidation and liver damage. Hence liver damage was reflected by high levels of serum AST, ALT, ALP and billirubin in $CCl_4$ treated rats. The rats treated with $CCl_4$ + extract showed low levels of AST, ALT, ALP and billirubin. One study reported that the ratio of AST/ALT greater than 1 in combination with a platelet count of less than 150,000 could predict advanced stage of fibrosis [26].

## Conclusion

In our study also the ratio of AST/ALT in $CCl_4$ treated rats was greater than 1 with the platelet count less than 150,000 in $CCl_4$ treated rats confirming the progression of fibrosis. Both these parameters were corrected towards normalcy in $CCl_4$ + extract treated rats. Hepatomegaly has also been reported as a symptom of cirrhosis. As cirrhosis has been defined as the end stage consequence of fibrosis and this symptom could be the reason for increase in the weight of liver noted in $CCl_4$ treated rats. In $CCl_4$ + extract treated rats the weight of liver was reduced and the liver/body weight ratio was also low. Moreover the architecture of the liver was less deranged in the $CCl_4$ + extract treated rats as shown in (Fig.2). These observations suggest that the *Lagerstroemia speciosa (L) pers.* extract is effective against $CCl_4$ induced liver fibrosis in rats.

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