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Hepatoprotective and Anticancer Potential of *Lagerstroemia speciosa* Acetone Leaf Extract in Experimental Rats

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ABSTRACT
This study investigates the hepatoprotective and anticancer effects of acetone leaf extracts from Lagerstroemia speciosa *(L.) Pers, in rats subjected to thioacetamide (TAA)-induced hepatotoxicity and carcinogen-induced tumour development. Experimental groups included a control, a TAA-treated group, a silymarin-treated group (standard drug), and groups treated with different doses of L. speciosa acetone leaf extract (ALE). Biochemical parameters such as SGOT, SGPT, ALP, and total bilirubin were analysed to assess liver function. Anticancer efficacy was evaluated based on body weight, liver weight, and histopathological alterations in liver tissues. TAA administration significantly elevated liver enzymes, indicating hepatocellular damage. Treatment with silymarin and ALE significantly improved liver function markers, with ALE at 500 mg/kg showing a dose-dependent protective effect. Additionally, ALE reduced tumour progression and improved liver histoarchitecture. The findings suggest that corosolic acid possesses both hepatoprotective and anticancer activities, highlighting its potential as a plant-based therapeutic candidate for liver disorders and cancer.*
Keywords: *Corosolic acid,* Lagerstroemia speciosa**,** *Silymarin, Hepatoprotective and Anticancer.*

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INTRODUCTION

Liver cancer is one of the leading causes of cancer-related mortality globally, with hepatocellular carcinoma (HCC) being the most prevalent primary liver malignancy. In India, cancer incidence is alarmingly high, with approximately 800,000 new cases and 550,000 deaths each year, contributing to the estimated 2.5 million cancer cases nationwide [1]. Chronic liver diseases, which often precede HCC, are fuelled by factors such as viral hepatitis (HBV, HCV), alcohol abuse, and drug-induced hepatotoxicity. According to the World Health Organization [2], nearly 10% of the global population is affected by chronic liver disorders, accounting for close to two million deaths annually.

Experimental hepatotoxic models play a crucial role in unravelling the pathogenesis of liver cancer. Thioacetamide (TAA), a well-established hepatotoxin, is widely used to induce hepatocellular damage, fibrosis, and carcinoma in animal models [3]. Metabolically activated in the liver, TAA generates reactive oxygen species (ROS) that initiate oxidative stress, centrilobular necrosis, and hepatocyte apoptosis, mimicking pathological conditions seen in human liver cancer. Due to its reproducibility and similarity to human liver pathology, TAA serves as a potent agent in experimental hepatocarcinogenesis [4].

Silymarin, a flavonoid complex extracted from *Silybum marianum*, has long served as the gold standard hepatoprotective drug. Its well-documented antioxidant, anti-inflammatory, and anti-fibrotic properties make it a benchmark against which other hepatoprotective agents are compared [5]. However, long-term use of synthetic drugs, including silymarin in certain formulations, may result in side effects such as gastrointestinal distress, allergic reactions, and drug interactions [6]. This has prompted the search for safer, plant-based alternatives with fewer adverse effects.

Medicinal plants offer a promising avenue for cancer prevention and therapy, especially within traditional systems such as Ayurveda and Siddha. More than 170 phytoconstituents from 110 plant species have demonstrated hepatoprotective activity [7]. These natural agents exert therapeutic effects by modulating

cellular pathways involved in inflammation, oxidative stress, and apoptosis. The use of herbal remedies is particularly advantageous due to their safety, availability, and cost-effectiveness [2].

Among these, *Lagerstroemia speciosa* (Banaba), an ornamental and medicinal tree native to tropical Asia, has gained attention for its bioactive compound corosolic acid. This pentacyclic triterpenoid exhibits potent antioxidant, anti-inflammatory, anti-diabetic, and hepatoprotective properties [8]. Corosolic acid has been shown to combat oxidative liver damage, regulate glucose metabolism, and suppress inflammation mechanisms that are crucial in mitigating the progression of liver diseases and hepatocellular carcinoma [9]. The growing body of evidence supports the exploration of *Lagerstroemia speciosa* as a viable natural agent for liver cancer prevention and management.

The experimental plant *L. speciosa* was selected for this study due to its rich phytochemical profile and traditional medicinal use in treating metabolic and inflammatory disorders. Notably, its major bioactive component, corosolic acid, has shown potent antioxidant, anti-inflammatory, and hepatoprotective properties in previous studies. Considering the limitations of conventional hepatoprotective agents like silymarin, including side effects and high cost, *L. speciosa* offers a promising natural alternative. Its ability to modulate oxidative stress and prevent liver damage makes it an ideal candidate for evaluating protective effects against thioacetamide (TAA)-induced hepatocellular carcinoma, a well-established animal model mimicking human liver pathology. Hence, this study aims to explore the efficacy of *L. speciosa* in mitigating liver damage and carcinogenesis.

MATERIAL AND METHODS

Chemicals and Reagents

Thioacetamide (TAA), silymarin, and other analytical-grade reagents were procured from Sigma-Aldrich, USA. All chemicals used were of the highest purity and analytical grade.

Collection and Preparation of Plant Extract

Fresh leaves of *L. speciosa* were collected from authenticated sources in Tamil Nadu, India. The leaves were washed, shade-dried, and powdered. Acetone extraction was performed using a Soxhlet apparatus with 70% acetone for 48 hours. The extracts were filtered, concentrated using a rotary evaporator, and stored at 4°C for further use [10].

Phytochemical Analysis

Phytochemical screening was performed to identify the presence of various bioactive compounds in the acetone extracts of *L. speciosa* leaves was performed using standard qualitative procedures to detect the presence of various secondary metabolites.

Alkaloids were detected using Wagner's and Dragendorff's reagents, which form reddish-brown and orange precipitates respectively in the presence of alkaloids [11].

Flavonoids were identified by the formation of a yellow color when treated with sodium hydroxide, which turns colourless upon acidification [12].

Phenols were estimated using the Folin–Ciocalteu reagent, resulting in a blue complex measurable spectrophotometrically [13].

Saponins were detected by the frothing method, in which persistent foam formation indicates their presence [14].

Tannins were identified by the formation of greenish-black or blue-black precipitates upon the addition of ferric chloride [15].

Steroids were screened using the Liebermann–Burchard test, where a green to blue color indicates the presence of steroidal nuclei [16].

The important compounds were identified from GC-MS analysis. The GC-MS analysis was conducted at The South Indian Textile Research Association, Coimbatore.

Experimental Animals

Healthy male Wistar albino rats (150–180 g) were procured from a certified animal house. The animals were acclimatized under standard laboratory conditions (temperature 25 ± 2°C, 12-hour light/dark cycle) and were provided with standard pellet diet and water ad libitum. All experimental procedures were approved by the Institutional Animal Ethics Committee of KMCH College of Pharmacy, Coimbatore (Approval No: KMCRET/ReRc/Ph.D/24/2021) and conducted according to CPCSEA guidelines.

Experimental Design

The animals were randomly divided into six groups (n=6 per group):

Group I (Normal Control) **:** Received distilled water.

Group II (TAA Control) **:** Received intraperitoneal injection of thioacetamide (200 mg/kg body weight).

Group III (Standard) **:** TAA + Silymarin (50 mg/kg orally, daily).

Group IV (Low) **:** TAA + ***L. speciosa*** Acetone Leaf Extract (ALE, 250 mg/kg orally, daily)

Group V (High) **:** TAA + ***L. speciosa*** Acetone Leaf Extract (ALE, 500 mg/kg orally, daily).

All treatments were administered for 12 weeks. The toxic group received a hepatotoxic dose of TTA (1 mL/kg body weight) once weekly, while the normal control group received an equivalent volume of saline.

Assessment of Hepatoprotective Activity

Biochemical Parameters

Blood samples were collected from the tail vein after the 14-day treatment period. Serum was separated and analysed for liver function markers, including alanine aminotransferase (SGPT), aspartate aminotransferase (SGOT), alkaline phosphatase (ALP), and total bilirubin [17, 18].

Histopathological Examination

Liver tissues were carefully excised, fixed in 10% formalin, and embedded in paraffin. Thin sections (5 µm) were prepared and stained with haematoxylin and eosin (H&E). The stained slides were examined under a light microscope to assess liver architecture, inflammation, necrosis, and regeneration.

Oxidative Stress Markers

Liver tissues were homogenized in cold phosphate buffer and analysed for oxidative stress indicators. Lipid peroxidation was measured by estimating malondialdehyde (MDA) levels. Antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT), were assessed [17, 18].

Statistical Analysis

All data were expressed as mean ± standard error mean (SEM). Statistical analysis was performed using one-way ANOVA followed by dunnett's test using GraphPad Prism. *P* value < 0.05 was considered statistically significant.

RESULTS

I. Phytochemical Analysis of *Lagerstroemia speciosa* Extract

The acetone leaf extract of *Lagerstroemia speciosa* contained several bioactive compounds. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenols, saponins, tannins, and steroids (Table 1). The bioactive compounds were identified from the GC-MS analysis of ALE shows in table 2.

Table 1: Phytochemical Analysis of *L. speciosa* Acetone Leaf extract

S.No	Phytochemicals	ALE
1	Carbohydrates	+
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Alkaloids	+
6	Glycosides	–
7	Cardiac Glycosides	+
8	Terpenoids	+
9	Triterpenoids	+
10	Phenols	+
11	Coumarins	+
12	Steroids	+
13	Phytosteroids	–

'+' Indicates the presence of active constituents
'-' Indicates the absence of active constituents

Table 2: Bioactive Compounds of *L. speciosa* Acetone Leaf extract from GC-MC

S.No	Retention Time	Compound Name	Molecular Formula	Match Factor
1	4.33	Benzene, 1,3 –dimethyl	C_8H_{10}	97.6
2	18.7	Naphthalene, 2,6- dimethyl	$C_{12}H_{12}$	97.5
3	3.96	2 - Pentanone,4-hydroxy - 4-methyl	$C_6H_{12}O_2$	97.1
4	24.92	Benzene, 1,1' - [1,2 - ethanediylbis(oxy)]bis	$C_{14}H_{14}O_2$	96.2
5	23.7	Pentadecanal	$C_{15}H_{30}O$	96.1
6	3.19	3 - Hexen - 2- one	$C_6H_{10}O$	96
7	24.59	6 - Hydroxy - 4,4,7a - trimethyl - 5,6,7,7a - tetrahydrobenzofuran -2(4H) - one	$C_{11}H_{16}O$	95.5
8	2.81	Toluene	C_7H_8	95.1
9	25.52	Neophytadiene	$C_{20}H_{38}$	94.5
10	17.53	1,1,5 - Trimethyl - 1,2 – dihydronaphthalene	$C_{13}H_{16}$	94.2
11	26.99	n - Hexadecanoic acid	$C_{16}H_{32}O_2$	94
12	3.55	2 - Pentanone, 4 – hydroxyl	$C_5H_{10}O_2$	93.6

13	25.24	Neophytadiene	$C_{20}H_{38}$	93.5
14	25.76	3,7,11,15 - Tetramethyl - 2 - hexadecen - 1 – ol	$C_{20}H_{40}O$	93.5
15	5.47	Benzene, (1 - methylethyl)	C_9H_{12}	93.2
16	21.18	2 - Butenedioic acid (Z) -, dibutyl ester	$C_{12}H_{20}O_4$	93.2
17	29.33	Naphthalene, 1 - (phenylmethoxy)	$C_{17}H_{14}O$	92.7
18	26.94	Phthalic acid, butyl hex - 3 - yl ester	$C_{18}H_{26}O_4$	92.6
19	24.96	Pentadecanal	$C_{15}H_{30}O$	92.5
20	22.11	Diethyl Phthalate	$C_{12}H_{14}O_4$	92.2
21	25.15	3,7,11,15 - Tetramethylhexadec - 2 - ene	$C_{20}H_{40}$	91.3
22	25.3	3,7,11,15 - Tetramethylhexadec - 2 - ene	$C_{20}H_{40}$	91
23	14.17	Benzofuran, 2,3 – dihydro	C_8H_8O	90.9
24	32.77	Hexadecanal	$C_{16}H_{32}O_2$	90.5
25	2.12	2 - Pentanone, 4 - hydroxy - 4 - methyl	$C_6H_{12}O_2$	90

II. Effect on Body and Liver Weight

A significant reduction in final body weight was observed in the TTA-treated toxicity group (Group II), where the weight dropped from 150.8 g to 88.2 g, indicating systemic toxicity. In contrast, the standard drug group (Group III) maintained body weight, with a slight increase from 146 g to 150.8 g. Rats treated with ALE extracts showed dose-dependent improvement in body weight. Group IV (ALE 250 mg/kg) showed a modest weight gain (149 g to 94 g), while Group V (ALE 500 mg/kg) showed a better recovery (147 g to 119.4 g), suggesting a protective effect against TTA-induced weight loss (Table 3).

Table 3: Effect of Acetone Leaf extracts of *L. speciosa* on Body Weight in Experimental Rats

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I	148.4 ± 1.43	157.6 ± 1.20
Group II	150.8 ± 2.59	88.2 ± 36.18
Group III	146 ± 1.23	150.8 ± 2.57
Group IV	149 ± 0.89	94 ± 38.39
Group V	147 ± 0.89	119.4 ± 30.12

*All the Values are expressed as mean ± SEM.

Table 4: Effect of Acetone Leaf extracts of *L. speciosa* on Liver Weight in Experimental Rats

Groups	Liver Weight (g)
Group I	3.563 ± 0.46
Group II	4.427 ± 0.46
Group III	3.38 ± 0.315
Group IV	3.75 ± 0.759
Group V	3.26 ± 0.92

*All the Values are expressed as mean ± SEM.

The liver weight was significantly increased in the TTA-treated toxicity group (Group II), measuring 4.427 g, compared to the normal control group (Group I) with 3.563 g, indicating liver enlargement due to hepatotoxicity. Treatment with the standard drug (Group III) and ALE at both doses (Groups IV and V) reduced the liver weight closer to normal. Group V showed the most notable reduction (3.26 g), suggesting effective hepatoprotection (Table 4).

III. Effect of Acetone Leaf extracts of *L. speciosa* on Liver Function Markers

In the TTA-induced toxicity group (Group II), there was a significant increase in liver enzymes and total bilirubin, indicating liver damage. SGPT and ALP levels were markedly elevated (189.5 U/L and 193.2 U/L, $P < 0.001$) compared to the normal control (Group I). Treatment with silymarin (Group III) and ALE extracts (Groups IV and V) led to notable reductions in liver enzymes. Group V showed significant improvement, with SGPT: 134.9 U/L, and total bilirubin: 0.52 mg/dL, approaching normal values, confirming hepatoprotective potential (Table 5).

Table 5. Effect of Acetone Leaf extracts of *Lagerstroemia speciosa* on Liver Function Markers in TTA-Induced Hepatotoxic Rats

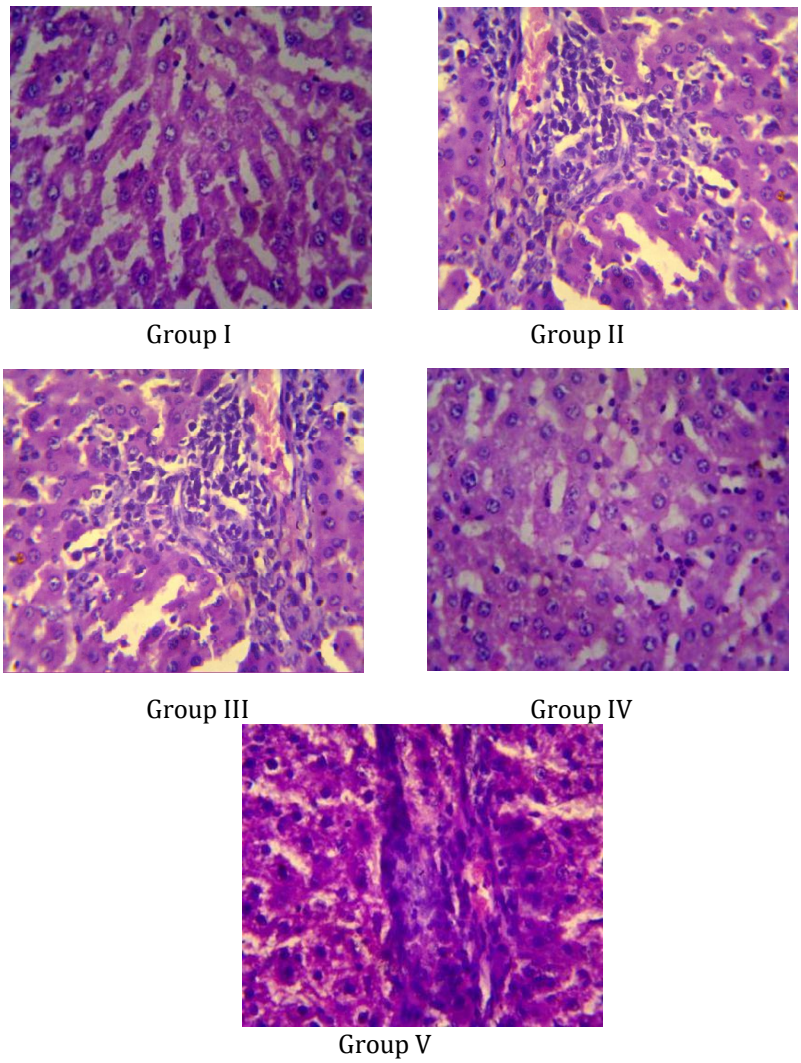
Groups	Total Bilirubin (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Group I	0.62 ± 0.24	48.4 ± 3.64	122.6 ± 3.47	136.8 ± 0.75
Group II	1.13 ± 0.33^{ns}	58.4 ± 5.25^{ns}	189.5 ± 3.48***	193.2 ± 2.84***
Group III	0.52 ± 0.02^{ns}	47.6 ± 2.59^{ns}	134.4 ± 1.24^{ns}	131.2 ± 1.39^{ns}
Group IV	0.75 ± 0.01^{ns}	35.05 ± 2.62^{ns}	148.9 ± 3.24***	139 ± 1.97^{ns}
Group V	0.52 ± 0.02^{ns}	71.55 ± 0.60*	134.9 ± 2.78*	130.3 ± 2.80^{ns}

Statistical comparison: Values are expressed as mean ± SEM Statistical significance (*P*) calculated by one way ANOVA followed by dunnett's (n=6); ns - Not Significant. P>0.05, **P*<0.05, ***P*<0.01, ****P*<0.001, calculated by comparing treated groups with control group.

IV. Histopathological Observations

Histological analysis of liver tissues stained with haematoxylin and eosin (H&E) revealed severe liver damage in the TTA-induced toxicity group. This group showed disrupted liver architecture, widespread necrosis, fatty changes, and heavy infiltration of inflammatory cells. In contrast, the liver sections from rats treated with silymarin (standard drug) and ALE (250 and 500 mg/kg) showed significant improvement. These groups displayed reduced necrosis, fewer inflammatory cells, and better-preserved liver structure. The corosolic acid-treated groups, particularly at the higher dose, exhibited more intact hepatocytes, indicating a clear hepatoprotective effect (Fig.1).

Fig. 1. Histological analysis of Liver tissue (H&E)



Group I Group II

Group III Group IV

Group V

V. Oxidative Stress and Antioxidant Enzyme Activity

The toxicity control group (Group II) showed significantly elevated total protein levels (1.08mg/dL), indicating high oxidative stress. Antioxidant enzyme activities, CAT (0.28umol/mg), were markedly reduced compared to the normal group (Group I). Treatment with silymarin (Group III) and ALE (Groups IV and V) significantly reduced SOD and LPO levels and improved antioxidant status. Notably, ALE at 500 mg/kg (Group V) restored SOD (0.21 unit/mg), CAT (0.35 umol/mg), and LPO (0.18 nmol/mg) levels close to normal, suggesting strong antioxidant and hepatoprotective effects (Table 6).

Table 6: Effect of *Lagerstroemia speciosa* Extracts on Oxidative Stress Markers and Antioxidant Enzyme Activities in Experimental Rats

Groups	Total protein (mg/dL)	SOD (unit/min/mg protein)	CAT (umol H_2O_2/min/mg protein)	LPO (nmol of MDA formed/mg protein)
Group I	0.83 ± 0.02	0.18 ± 0.002	0.38 ± 0.05	0.17 ± 0.005
Group II	1.08 ± 0.12**	0.38 ± 0.02***	0.28 ± 0.02	0.40 ± 0.01***
Group III	0.66 ± 0.053	0.21 ± 0.016	0.46 ± 0.05	0.15 ± 0.012
Group IV	0.85 ± 0.03*	0.26 ± 0.006**	0.30 ± 0.024	0.23 ± 0.009*
Group V	0.62 ± 0.044	0.21 ± 0.009	0.35 ± 0.014	0.18 ± 0.008

Statistical comparison: Values are expressed as mean ± SEM Statistical significance (*P*) calculated by one way ANOVA followed by dunnett's (n=6); ns - Not Significant. *P*>0.05, **P*<0.05, ***P*<0.01, ****P*<0.001, calculated by comparing treated groups with control group.

DISCUSSION

The present study evaluated the hepatoprotective potential of *Lagerstroemia speciosa* ethanolic leaf extract, particularly its active compound corosolic acid, against TTA-induced liver toxicity in rats. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins, and steroids (Table 1), [19].

The liver function test results demonstrated that the TTA-treated group (Group II) showed a significant elevation in serum biomarkers such as ALT, AST, ALP, and total bilirubin, along with a reduction in albumin, indicating hepatic damage. ALE 500 mg/kg (Group V), significantly normalized these parameters, showing a hepatoprotective effect comparable to the standard drug silymarin (Table 5). This finding is consistent with earlier studies that reported liver enzyme modulation by plant-based antioxidants [20].

Histopathological evaluation confirmed severe hepatic necrosis and inflammation in the toxic group, whereas both doses of corosolic acid reduced hepatic cell damage, restored normal architecture, and reduced inflammatory infiltration.

Regarding oxidative stress, rats in the TTA group showed increased total protein and LPO levels and decreased antioxidant markers (SOD and CAT), indicating lipid peroxidation and oxidative damage. *L. speciosa* extracts significantly reversed these effects by lowering MDA and enhancing antioxidant enzyme activities, suggesting its antioxidant role in hepatoprotection (Table 6).

The body weight and liver weight assessments also reflected the protective action of corosolic acid. TTA caused significant weight loss and liver enlargement, which were improved in the treated groups. These physiological improvements corroborate the biochemical and histological findings.

CONCLUSION

The findings of this study strongly indicate that corosolic acid, a major compound in *Lagerstroemia speciosa*, exerts significant hepatoprotective and antioxidant effects against TTA-induced liver toxicity in rats. Its efficacy was demonstrated through improvements in liver enzyme profiles, oxidative stress parameters, histopathological architecture, and overall physiological conditions.

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Conflict of Interest

There are no conflicts of interest.

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