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Evaluation of the Antioxidant Properties of Flavonoids and Polyphenols in Acetone Leaf Extract of *Lagerstroemia speciosa* against DEN-induced Toxicity in Albino Rats

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Abstract

Hepatocellular carcinoma is a prevalent malignancy globally, posing a significant challenge to healthcare systems. Severe liver damage can result from minimal exposures to diethyl nitrosamine (DEN) or dimethyl nitrosamine (DMN) via parenteral or oral routes. DEN, available commercially for experimental use, is also present in tobacco smoke, water, cured and fried foods, agricultural chemicals, cosmetics, and pharmaceuticals. *Lagerstroemia speciosa*, known as India's pride, is the focus of this study. The main objective is to evaluate the anti-cancer potential of experimental plant extracts against DEN-induced liver tumours in albino rats. Phytochemical analysis confirms the presence of various compounds in the *L. speciosa* acetone leaf extract (LALE), including proteins, amino acids, reducing sugars, glycosides, flavonoids, saponins, phenols, tannins, and coumarins. Groups IV and V received different doses of LALE. Group III received intraperitoneal 5-fluorouracil twice weekly for 28 days after DEN induction. Group II served as the DEN-induced negative control, while the control group received saline alongside a standard diet. Results indicated morphological changes, increased liver weight, decreased hepatic enzymes, elevated levels of GGTP, GPx, GST, and LPO, and decreased levels of SOD and catalase. These findings collectively demonstrate a significant anti-cancer effect of acetone leaf extracts from *Lagerstroemia speciosa.*

***Key words*:** Hepatocellular carcinoma, DEN, *Lagerstroemia speciosa*, Hepatic enzymes

India is recognized as the world's largest producer of medicinal plants and is often referred to as the "Botanical Garden of the world." In traditional medicine systems, Indian medicinal plants are highly valued for their bioactive compounds, which serve as the foundation for many modern pharmaceuticals. These plants not only provide essential healthcare for rural communities but also attract scientific interest due to their potential in drug discovery and development [1]. Leafy greens contain numerous phytochemicals that exhibit antioxidant properties. Antioxidants play a crucial role in protecting cells from damage caused by free radicals generated during oxidative metabolism [2]. It is essential for antioxidants to integrate with cellular metabolic processes to function effectively, undergo metabolism, and be eliminated from the body. While the chemical characteristics of antioxidants are well-understood [3], there remains limited knowledge about their actual physiological effects and metabolic activities. Several bioactive compounds with strong antioxidant properties, including glycosides, flavonoids, saponins, phenols, and tannins, are present in the secondary metabolites of medicinal plants [4]. These compounds contribute to enhancing both non-specific and specific immune responses, thereby potentially boosting the body's ability to resist infections and restore internal balance within tissues. Consequently, the use of traditional medicines is widely acknowledged for reducing the risk of various types of cancer [5].

Lagerstroemia speciosa (L.), commonly known as "Jarul" in India, belongs to the Lythraceae family. It is also referred to as Pride of India and Queen's Flowers, or Queen Crape Myrtle in English. This plant is native to Southeast Asian countries, including the Philippines and India. Pharmacologically active phytochemicals isolated from different parts of *L. speciosa* have demonstrated antibacterial, hypoglycaemic, anti-inflammatory, hepatoprotective, and antioxidant properties [6-7].

The liver possesses remarkable regenerative abilities, capable of repairing mild to moderate damage with time and appropriate care. However, severe or chronic damage can lead to complications such as liver failure, cirrhosis (scarring of the liver), and an increased risk of liver cancer. Liver diseases, or hepatopathies, have emerged as significant causes of illness and death in both humans and animals [8]. These conditions arise from various factors including viral infections, bacterial infections, fungal toxins (such as aflatoxins), malnutrition, alcohol abuse, chemical imbalances in the body, and autoimmune disorders [9]. Liver damage typically involves cellular necrosis, increased lipid peroxidation in tissues, oxidative damage, and fluctuations in biochemical markers such as SGOT, SGPT, triglycerides, cholesterol, bilirubin, and

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alkaline phosphatase [10]. The objective of the study is to assess the antioxidant properties of flavonoids and polyphenols found in acetone leaf extracts of *Lagerstroemia speciosa* in countering DEN-induced toxicity in albino rats.

MATERIALS AND METHODS

Collection and authentication of experimental plant

The leaves of *L. speciosa* were gathered from the PG Girls Hostel, Government Arts College (Autonomous), located in Coimbatore District, Tamil Nadu, India. The plant was identified and confirmed authentic at the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore-03 (Identification No. BSI/ SRC/ 5/ 23/ 2020/ Tech/ 52).

Experimental plant extract

The leaves of the experimental plant were meticulously cleaned before preparing the extract. Subsequently, the samples were dried in the shade at room temperature (27± 2ºC) for approximately two weeks until completely dried. The dried leaves were finely ground using a blender. A hundred grams of the powdered plant material was immersed in 1000 ml of acetone within a sealed wide-mouthed container and left for four days with intermittent shaking. Following this, the extract was filtered using Whatman No. 1 filter paper and then dried in a plastic tray at room temperature [11].

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the green leaf extracts of *L. speciosa* using acetone was conducted following the methodologies outlined by Harborne [12] and Trease and Evans [13]. Additionally, GC-MS analysis was performed at The South Indian Textile Research Association in Coimbatore to identify key compounds present in the acetone extracts of *L. speciosa* leaves. The analysis utilized Thermo GC-Trace Ultra ver. 5.0 and Thermo MS DSQ II chromatography [14].

Acute oral toxicity studies

The acetone leaf extracts were given orally to three groups, each consisting of six animals, and their behaviour was closely observed for any noticeable changes. A toxicological evaluation conducted over a 7-day period confirmed no instances of mortality. Consequently, it was concluded that proceeding with the experiment was safe based on these results.

Experimental animal

Male Wistar rats aged 2 months and weighing between 100 to 150 g were selected for the study. They were housed in the animal facility at a room temperature of 22°C with a 12-hour light/12-hour dark cycle. The rats had ad libitum access to commercial pellet diet and water throughout the study period. All experimental procedures were conducted following approval from the Institutional Animal Ethics Committee (IAEC) of KMCH College of Pharmacy, Coimbatore (Approval No. KMCRET/ReRc/ Ph.D./25/2021).

Experimental design

The experimental animals were divided into five groups for the study. Group I served as the control group, Group II consisted of animals induced with DEN (di ethyl nitrosamine), Group III included animals induced with DEN and treated with a standard drug (STD), and Group IV and V were induced with DEN and treated with *L. speciosa* acetone leaf extracts at low and high doses (250 mg/kg body weight and 500 mg/kg body weight) daily for 16 weeks.

Group I: Control group received only normal saline throughout the experiment and served as the control group.
Group II: Animals received DEN.
Group III: Animals received DEN and STD (standard drug) (20mg/kg) twice a week for 28 days.
Group IV: Animals received DEN and LALE (*L. speciosa* acetone leaf extract) at a low dose orally (250 mg/kg).
Group V: Animals received DEN and LALE (*L. speciosa* acetone leaf extract) at a high dose orally (500 mg/kg).

Determination of ex-vivo antioxidant activity

The study examined liver antioxidants via hepatic isolation, organ dissection, and animal euthanasia, assessing parameters such as total protein [15], superoxide dismutase [16], catalase [17], glutathione peroxidase [18], reduced glutathione [19], and lipid peroxidation [20].

Antioxidant and biochemical parameters

At the end of the experiment, blood samples of 10 ml each were drawn from the jugular vein of each animal into vacuum glass tubes without anticoagulant. After allowing the tubes to stand at room temperature for 20 minutes, they were centrifuged at 3,000 rpm for 10 minutes. The resulting serum samples were then stored at -25°C until analysis.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Statistical significance (P) was determined using one-way ANOVA followed by Dunnett's posts hoc test. "ns" indicates no significance, while $^{**}P < 0.05$ denotes statistical significance when compared to the control group.

RESULTS AND DISCUSSION

Result 1: Qualitative phytochemical analysis

The qualitative phytochemical analysis identified the presence of several active compounds in the acetone extract of *L. speciosa* leaves (LALE), including alkaloids, flavonoids, saponins, phenols, tannins, proteins and amino acids, reducing sugars, glycosides, phytosterols, and coumarins.

Result 2: Acute toxicity studies

Administration of acetone leaf extracts of *L. speciosa* at a dose of 500 mg/kg to rats during an acute toxicity test showed no signs of toxicity, mortality, or morbidity. Furthermore, there were no significant alterations observed in behaviour or gait.

Result 3: Liver weight

Control Group: Liver weight increase is indicated as 4.13%. Group II (Only DEN + PB): Liver weight increase is significantly higher at 58.03% compared to the control group. Group III (DEN + STD): Liver weight increase is 14.85%, which is also higher compared to the control. Group IV (DEN + Ext 250 (mg/kg)): Liver weight increase is 32.30% compared to the control. Group V (DEN + Ext 500 (mg/kg)): Liver weight increase is 56.20% compared to the control.

Result 4: In-vivo antioxidant activity

Treatment with *L. speciosa* acetone leaf extracts (EXT) at both low dose (250 mg/kg L.D.) and high dose (500 mg/kg H.D.) resulted in varied effects on antioxidant enzymes, with some changes observed but not reaching statistical significance compared to the control group. These results suggest that *L. speciosa* acetone leaf extracts may exert antioxidant effects, even though not as potent as the standard drug in this experimental setup.

Result 5: Ex-vivo antioxidant activity

The administration of DEN (di ethyl nitrosamine) combined with phenobarbital (DEN + PB) significantly increased levels of SOD, catalase, GPX, GSH, and LPO compared to the control group, indicating oxidative stress and liver damage. Treatment with the standard drug (STD) showed varying effects on antioxidant enzymes, with significant increases observed in SOD and catalase.

Table 1 Ex-vivo antioxidant activity of acetone leaf extracts of *Lagerstroemia speciosa*

Groups	Control	Only DEN+ PB	DEN+ STD	DEN+LALE 250mg/kg L. D	DEN+LALE 500mg/kg H. D
Total protein (mg/dl)	0.912± 0.123	1.25± 0.0696^{ns}	0.745± .0948^{ns}	0.843± 0.0638^{ns}	0.799± 0.0648^{ns}
SOD (Unit/min/mg protein)	0.0727±0.00273	0.0923±.00203**	0.0443±.00448***	0.0577± 0.0318*	0.0493±.00203***
CATALASE (µmol/H_2O_2 /consumed/ min/mg protein)	0.0367± 0.00291	0.0767±0.00581***	0.0333±0.00353^{ns}	0.0423± 0.00376^{ns}	0.0363± 0.00639^{ns}
GPX (nmol of Glutathione oxidase/min/mg protein)	0.034± 0.00306	0.0837±0.00754**	0.0273±0.00521^{ns}	0.037± 0.0131^{ns}	0.0263± 0.00384^{ns}
GSH (nmol/min/mg protein)	0.0503± 0.00722	0.0907±0.00219**	0.0313±0.00694^{ns}	0.055± 0.00889^{ns}	0.0353± 0.00333^{ns}
LPO (nmol of MDA/mg protein)	0.113± 0.00794	0.247±0.0121**	0.127±0.00601^{ns}	0.156± 0.00586^{ns}	0.139± 0.00321^{ns}

The data are presented as mean ± standard deviation (SD)

Statistical significance (*P*) was determined using one-way ANOVA followed by Dunnett's posts hoc test

SOD is an enzyme that catalysis the dismutation of superoxide radicals into hydrogen peroxide and oxygen, playing a crucial role in antioxidant defence. The increase in SOD activity in DEN + PB and *Lagerstroemia speciosa* extract-treated groups suggests an adaptive response to oxidative stress induced by DEN. However, the decrease in SOD activity in the DEN + STD group may indicate compromised antioxidant defence mechanisms. Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen, contributing to antioxidant defence. The significant increase in catalase activity in the DEN + PB group suggests an enhanced response to oxidative stress. The comparable catalase activities in the DEN + STD and *Lagerstroemia speciosa* extract-treated groups indicate potential variations in antioxidant responses under different treatment conditions.

GPX is an enzyme that reduces hydrogen peroxide and organic hydroperoxides using glutathione as a cofactor, thereby protecting cells from oxidative damage. The significant increase in GPX activity in the DEN + PB group suggests an upregulation of antioxidant defence mechanisms. The relatively stable GPX activities in the DEN + STD and *Lagerstroemia speciosa* extract-treated groups suggest potential differences in oxidative stress responses.

Reduced glutathione (GSH) is a critical antioxidant that scavenges reactive oxygen species and maintains redox homeostasis. The increase in GSH activity in the DEN + PB group suggests an enhanced antioxidant response. The variability in GSH activities in the DEN + STD and *Lagerstroemia speciosa* extract-treated groups may reflect different levels of oxidative stress and antioxidant capacity.

Lipid peroxidation (LPO) is a marker of oxidative damage to cell membranes, resulting from the reaction of reactive oxygen species with lipids. The significant increase in LPO level in the DEN + PB group indicates oxidative stress-induced lipid damage. The stable LPO levels in the DEN + STD and *Lagerstroemia speciosa* extract-treated groups suggest potential protective effects against lipid peroxidation.

The significant increases in liver weight observed in the DEN-treated groups (DEN + PB, DEN + STD, DEN + Ext) indicate hepatomegaly, which is often associated with liver damage or metabolic changes induced by DEN. DEN combined with phenobarbital (PB) resulted in the highest increase in liver weight (58.03%), suggesting severe liver damage. The standard drug (STD) and *Lagerstroemia speciosa* acetone leaf extracts at both doses also contributed to liver weight, even though to a lesser extent compared to DEN + PB.

The *Lagerstroemia speciosa* acetone leaf extracts contain various bioactive compounds, which may influence liver function and metabolism. The dose-dependent response observed with the extracts suggests that higher concentrations may have more pronounced effects on liver weight. These findings highlight the importance of evaluating the impact of natural extracts and standard drugs on liver health in experimental models. Further studies would be necessary to understand the mechanisms behind these observed changes and to determine if the effects translate to potential therapeutic benefits or risks in clinical settings.

CONCLUSION

In conclusion, while the *Lagerstroemia speciosa* acetone leaf extracts showed some modulation of liver weight compared to DEN-induced damage, the significant increases in liver weight across all treated groups underscore the hepatotoxic effects of DEN and the need for effective therapeutic interventions in liver disease research. This study provides valuable insights into the antioxidant properties of *Lagerstroemia speciosa* and highlights its potential as a therapeutic agent in combating oxidative stress-related liver damage.

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