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EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF ROOTS OF *LAGERSTROEMIA SPECIOSA* PERS.

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ABSTRACT

Lagerstroemia speciosa pers roots known as Banaba (Jarul) (Family-Lythraceae), was subjected to investigate the hepatoprotective and antioxidant activity of alcoholic extract of roots of *Lagerstroemia speciosa* pers against CCl₄ induced hepatotoxicity. Preliminary screening of alcohol extract showed presence of Phenolic compound, flavanoids and saponins. Administration of alcoholic extract produced significant hepatoprotective effect as demonstrated by decrease level of serum liver marker enzymes like AST, ALT, ALP, SBRN and increase protein level. It also showed antioxidant activity by increase in activity of GSH, SOD, CAT and decrease in TBARS level compare to CCl₄ treated group. A comparative histopathological study of liver exhibited almost normal architecture, as compared to CCl₄ treated group. It can be concluded *Lagerstroemia speciosa* pers roots that protect hepatocytes from CCl₄-induced liver damages due to its antioxidant effect on hepatocytes.

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Key Words

Lagerstroemia speciosa Pers.,
Carbon Tetrachloride,
Hepatoprotective, Antioxidant.

INTRODUCTION

Lagerstroemia speciosa (L) Persoon (syn. *L. flos-reginae* Retz.) is a medium-sized to large deciduous tree with a rounded crown. It is commonly known as Jarul in Hindi, Arjuna in Sanskrit and Crape Myrtle in U.S.A. and is distributed more or less throughout in Assam, Bengal, the Deccan Peninsula,^[1] Chittagong, Lower Burma, Malay Peninsula, foot of Western Ghats up to 2000 feet, Ceylon and Java.^[2] The leaves of *Lagerstroemia speciosa* are purgative, deobstructive and diuretic. A decoction of the leaves prepared like tea is used for diabetes mellitus in Philippines.^[3] The role of free radicals and antioxidants in the pathogenesis of human diseases and in the process of ageing has led to the suggestion that antioxidants, in particular plant derived antioxidants, may have health benefits as prophylactic agents.^[4] It is also used for abdominal pains, mouth ulcers, roots are considered astringent, stimulant and febrifuge.^[5] The leaves extract of this plant is reported earlier as an antioxidant and as nephroprotective agent^[6] and also reported as an hepatoprotective and free radical scavenging activity.^[7]

Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicines in India.^[8] Hepatic fibrosis is a common condition in which major amounts of liver parenchyma cells are replaced by fibrous connective tissue. Liver diseases remain one of the serious health problems and it is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury.^[9] Experimentally hepatic fibrosis is formed by the administration of CCl₄, paracetamol, thioacetamide etc. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues. It has been stated that one of the principal causes of CCl₄ induced hepatopathy is lipid peroxidation by CCl₃, a free radical derivative of the toxin.^[10] In this study we report on the protective effect of *L. speciosa* alcohol extract against hepatotoxin CCl₄. The literature reviews indicated the hepatoprotective activity of *L. speciosa* leaf ethanol extract, but the roots of *L. speciosa* has been used as a folk medicine for jaundice. However there are no scientific bases or reports in the

modern literature regarding its usefulness as hepatoprotective agent evaluated so far. Hence the present study has been undertaken to investigate the antioxidant and hepatoprotective activity of roots alcohol extracts of *L. speciosa* CCl₄ induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material

The plant of *L. speciosa* was collected from places Kamdhenu foods, Matruchaya, Mulla Wadi, Valsad, Gujarat state, India. Plant was identified and authenticated by Dr. Minoo. H. Parabia (F.E.S., F.I.A.T) Shri Bapalal Vaidya Botanical Research Centre, Department of Biosciences, Veer narmad south Gujarat University, Surat and voucher specimens was kept in department of Pharmacognosy, APMC College of Pharmaceutical Education and Research, Himatnagar. The powdered leaves were subjected to successive soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether, Benzene, Chloroform, Acetone, ethanol and water respectively. The extracts were vacuum dried and the percentage yields of the extracts were 0.62, 5.08, 5, 2, 5 and 2.15% respectively.

Animals

Healthy untreated Wistar rats of either sex (equal ratio) weighing 180–250 g (16–18 weeks old) were used for hepatoprotective activity and Swiss albino female mice weighing between 25 and 30 g (10–12 weeks old) were used for acute toxicity study. All animals (mice and rats) were collected from the animal house, Zydus Cadila Pharmaceuticals, Ahmedabad. The animals were grouped and housed in polyacrylic cages with not more than two animals per cage and maintained under well-controlled

conditions of temperature (27 ± 2°C), humidity (55 ± 5%), and 12/12 h light–dark cycle. Conventional laboratory diet and tap water were provided *ad libitum*. The protocol of the experiments was approved by the Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of

Social Justice and Empowerment, Government of India (IAEC Proposal no. APMC 11/04, Himatnagar).

Phytochemical screening:

The dried extract of roots was subjected to the preliminary phytochemical analysis for the presence of different phytoconstituents.^[11,12]

Acute toxicity study

The animals (mice) were divided into seven groups of six animals each. The control group received normal saline (2 mL/kg body weight p.o.) while other groups received 300, 600, 800, 1000, 2000, and 3000 mg/kg of the test extract. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioral changes. They were then kept under observation for 14 days after drug administration to find out the mortality if any. Observations were made twice daily, one at 7 a.m. and again at 7 p.m.^[13]

Hepatoprotective activity of the extract against acute CCl₄ toxicity

The animals were divided into five groups each consisting of six animals. Carbon tetrachloride diluted with olive oil (1:1) was administered (2.5 ml/kg, p.o.), treatment with *L.speciosa* root alcohol extract and Liv-52 except control to induced hepatotoxicity. The control group of rats received 1% w/v acacia mucilage (1 ml/kg, p.o.) three times at 12 h intervals and olive oil (1.25 ml/kg, p.o.) 30 min after the administration of first dose of acacia mucilage. The CCl₄ group of rats received 1% w/v acacia mucilage (1ml/kg, p.o.) 3 times in 12 h intervals and a single dose of CCl₄ solution (2.5 ml/kg, p.o.) 30 minutes after first dose of acacia mucilage. The test groups received test suspensions by forage three times at 12 h intervals. CCl₄ (2.5 ml/kg, p.o.) was administered 30 minutes after the first dose of test suspensions. 36 h after CCl₄ administration, blood was collected from all groups of rats by puncturing retro orbital plexus.^[14]

Group I: Normal control animals (received vehicle),

Group II: Carbon tetrachloride treated control animals,

Group III: *L.speciosa* root alcohol extract treated animals (100 mg/kg b.w, p.o 3 times at 12 h interval).

Group IV: *L.speciosa* root alcohol extract treated animals (200 mg/kg b.w, p.o 3 times at 12 h interval).

Group V: Liv 52 treated animals (100 mg/kg b w, p.o 3 times at 12 h interval).

Biochemical studies

The blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 minutes and analysed for various biochemical parameters Aspartate transaminase (AST), Alanine transaminase (ALT),^[15]Alkaline phosphatase (ALP),^[16]bilirubin^[17] and total protein^[18].Animal was sacrificed and liver was isolated and used for Histopathological analysis.^[19]

Antioxidant activity

For estimating antioxidant activity, animals were sacrificed and liver was excised, rinsed in ice-cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10 % w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation (TBARS).^[20]A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of glutathione.^[21]The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of super oxide dismutase^[22] and catalase.^[23]

Histopathological studies

Paraffin sections of buffered formalin-fixed liver samples were stained with hematoxyline-eosin to study the histological structure of control and treated (Toxicant, *L.speciosa* roots extract, Liv-52) rats liver.

Statistical analysis

Results are expressed as mean \pm S.E.M. The statistical difference was analyzed by one way analysis of variance followed by Tukey-Kramer multiple comparison test, significance was calculated as the P value and P values of less than 0.05 were regarded as statistically significant.

RESULTS

Preliminary phytochemical screening showed the presence of tannins, flavonoids and saponins in *L.speciosa* pers roots. (Table1)

Table 1. Qualitative phytochemical evaluation of the Lagerstroemia speciosa extracts Observation

Sr. No	Phytochemical Nature	Petroleum ether extract	Benzene extract	Ethanol extract	Water extract
1	Flavanoids	–	–	+	+
2	Tannins (Phenolic compounds)	–	–	+	+
3	Saponins	–	–	–	+

'+' and '–' indicates the presence and absence of the active constituents.

In acute toxicity study, it was observed that there was no mortality at any of the tested doses up to end of 14 days of observation.

Hepatic damage induced by CCl₄ caused significant increase (P<0.01) in marker enzymes ALT, AST, ALP and bilirubin levels as compared to normal animals. CCl₄ toxicity also showed significant decrease (P<0.01) in protein level compare to normal animal. Oral

administration of alcoholic extract of *L.speciosa* roots (100 mg/kg) significantly (P<0.001) lowered ALT, AST and ALP compare to CCl₄ treated rats. *L.speciosa* roots treatment also showed significant decrease (P<0.001) in bilirubin levels compare to CCl₄ treated animals. Level of serum protein was also significantly (P<0.001) increased in rats, which received *L.speciosa* roots as compared to CCl₄ treated group (Table 2).

Table 2. Effect of drug extract on various hepatic biochemical parameters in CCl₄ induced hepatotoxicity.

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Bilirubin (mg%)	Total Protein (gm/dl)
Control	34.17 ± 1.33	91.33 ± 3.19	27.67 ± 2.30	0.49 ± 0.04	7.03 ± 0.17
Toxicant	83 ± 2.76*	816 ± 10.31*	131.67 ± 2.99*	4.73 ± 0.23*	3.82 ± 0.19*
<i>L.speciosa</i> 100 mg/kg	40.83 ± 1.66**	333.00 ± 29.25**	116.00 ± 2.84***	1.11 ± 0.03**	4.24 ± 0.21 ^{NS}
<i>L.speciosa</i> 200 mg/kg	35.00 ± 1.53**	202.67 ± 23.37**	41.33 ± 1.93**	0.69 ± 0.02**	5.90 ± 0.21**
Liv-52	34.67 ± 1.90**	198 ± 3.28**	37.83 ± 2.97**	0.55 ± 0.03**	6.68 ± 0.11**

Values are mean ± SEM of 6 animals in each group

*P<0.001 relative to Normal group, **P<0.001 relative to Toxicant group, ***P<0.01 relative to Toxicant group, NS non significant

Hepatic damage induced by CCl_4 also caused significant ($P < 0.001$) increase in thiobarbituric acid reactive substance (TBARS) level and significantly ($P < 0.001$) decrease the activity of antioxidant enzymes SOD and CAT in liver when compared with normal rats (Table 3). Intracellular antioxidant GSH level was also significantly ($P < 0.001$) depleted. Treatment with *L.speciosa* roots significantly ($P < 0.001$) prevented the increase in TBARS

levels and brought them near to normal levels compared to CCl_4 treated rats. *L.speciosa* roots treatment also showed significant ($P < 0.001$) increase in GSH activity. SOD and CAT activity were significantly ($P < 0.001$) increased in *L.speciosa* roots treated groups compare to CCl_4 treated group. The effects of *L.speciosa* roots were comparable to that of standard reference drug Liv-52.

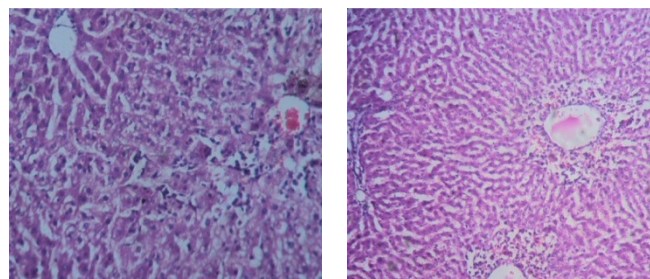
Table 3:- Effect of drug extract on TBARS, SOD, CAT and GSH level in CCl_4 induced hepatotoxicity.

Group	TBARS (nmol/mg protein)	SOD (U/mg protein)	Catalase (U/min/mg protein)	GSH (nmol/mg protein)
Control	2.20 ± 0.05	16.48 ± 0.90	17.47 ± 0.48	22.02 ± 0.89
Toxicant	$4.99 \pm 0.11^*$	$4.47 \pm 0.18^*$	$8.55 \pm 0.33^*$	$10.90 \pm 0.92^*$
<i>L.speciosa</i> 100mg/kg	$3.14 \pm 0.11^{**}$	$7.77 \pm 0.41^{**}$	$10.63 \pm 0.20^{***}$	11.47 ± 0.79^{ns}
<i>L.speciosa</i> 200 mg/kg	$2.49 \pm 0.07^{**}$	$8.72 \pm 0.14^{**}$	$13.93 \pm 0.40^{**}$	$19.26 \pm 0.44^{**}$
Liv-52	$2.32 \pm 0.05^{**}$	$10.63 \pm 0.22^{**}$	$14.83 \pm 0.47^{**}$	$19.35 \pm 0.31^{**}$

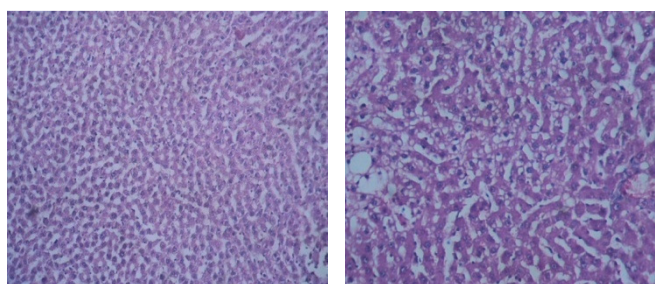
Values are mean \pm SEM of 6 animals in each group

* $P < 0.001$ relative to Normal group, ** $P < 0.001$ relative to Toxicant group, *** $P < 0.01$ relative to Toxicant group, NS non significant

Histopathological study of CCl_4 treated rat livers showed marked necrosis, inflammation, lymphocytic infiltration, severe fatty degeneration and extensive vacuolization with disappearance of nuclei compared to normal untreated rats. (Fig1a, 1b) *L.speciosa* roots and Liv-52 treated liver were structurally normal as compare to the CCl_4 treated group (Fig 1c, 1d).

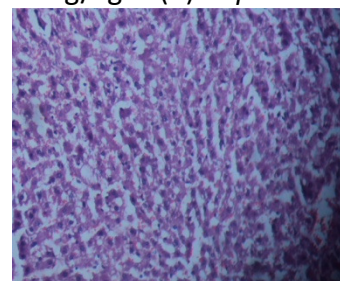


(c) *L.speciosa* 100mg/kg (d) *L.speciosa* 200mg/kg



(a) Control

(b) Toxicant



(e) Liv-52

Fig 1:- Photomicrograph of liver section taken from rats against CCl_4 induced hepatotoxicity (10X)

a: Normal control rat showed hepatic cells with nuclei, cytoplasm, central vein and portal triad.

b: olive oil: CCl₄ treated rats showed marked necrosis, inflammation, lymphocytic infiltration severe.

c: LS ethanol extract (100mg/kg) treated rats showed necrosis, inflammation, lymphocytic infiltration and occasional regenerating parenchymal cells.

d: LS ethanol extract (200mg/kg) treated rats showed mild inflammatory changes and greater area of regeneration.

e: Liv-52 (100 mg/kg) treated rats showed mild inflammatory changes and greater area of regeneration.

DISCUSSION

Pretreatment of rats with *L. speciosa* extract dose dependently inhibited the increased level of all hepatic marker enzymes in serum, indicating the liver protective activity of *L. speciosa*. Stabilization of serum total bilirubin and total protein levels by the pre administration of *L. speciosa* extract to rats, dose dependently for 3 times at 12h interval prior to the CCl₄ administration is a clear indication of the improvement of functional status of the hepatic cells.^[24] The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminase and ALP was the clear indications of cellular leakage and loss of functional integrity of cell membrane.^[25] The hepatotoxicity by CCl₄ is a result of reductive dehalogenation, which is catalyzed by cytochrome P - 450 and forms the highly reactive trichloromethyl free radical (CCl₃-). This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical (CCl₃OO-).^[26] This also forms covalent bond with sulphhydryl group of several membrane molecules like glutathione, which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction.^[27] Both radicals are capable of binding to proteins or lipids or of abstracting a hydrogen atom from a unsaturated lipids, which initiate lipid peroxidation and liver damage and by play a significant role in the pathogenesis of diseases.^[28]

The lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity induced by toxins.^[29] Lipid peroxidation is viewed as a complicated

biochemical reaction involving free radicals, oxygen, metal ions and they host of other factors in the biological system. Since a lipid constitutes nearly 60% of the compounds in biomembranes, only major perturbation is bound to affect structure and function of the cell. The extract demonstrated potent superoxide and other free radical scavenging property.^[6] Therefore it may be inferred that anti oxidant property of the extract may prevent the formation of trichloro methyl peroxide radical. Thereby inhibit the lipid peroxidation and offer hepatoprotection against CCl₄ challenge. Extent of decrease in tissue GSH and tissue lipid peroxidation is a measure of tissue destruction.^[30] Prevention of tissue GSH depletion by the extract treatment also indicates the natural inbuilt tissue protective mechanism is kept intact and oxidative degeneration of tissue is prevented.

Alteration in the activity of alkaline phosphatase may be due to the disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes after CCl₄ administration. Erythrocytes are regularly subjected to high oxygen tension as they are among the first cells exposed to exogenous oxidative substance that are ingested, injected or inhaled.^[31]

Plant constituents like triterpenoids and flavonoids are well known for their antioxidant and hepatoprotective activities.^[7] The phytochemical analysis of ethanol extract of *L. speciosa* revealed the presence of saponins, tannins, saponins and flavonoids etc. The present findings provide pharmacological evidence to the ethnomedicinal property of *L. speciosa* in treating acute jaundice.

In conclusion, the results of this study demonstrate that LS root has potent hepatoprotective action upon Paracetamol and carbon tetrachloride induced hepatic damage in rats. Our result showed that the hepatoprotective effect of LS root may be due to its antioxidant and free radical scavenging properties. Hepatoprotective activity of LS root may be due to presence of Tannin. Further, investigation is underway to determine phytoconstituents which is responsible for its hepatoprotective effect.

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