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Research Article

ANTICALCIURIA EFFECT OF METHANOLIC ROOT EXTRACT OF *LAGERSTROEMIA SPECIOSA* (L). PERS (LYTHRACEAE) AGAINST HIGH PROTEIN DIET INGESTED IN ALBINO RATS

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ABSTRACT
Nephrolithiasis, commonly known as kidney stone disease or urolithiasis, is a crystallopathy in which a solid mass forms in the urinary tract. The aim of the present research is to evaluate the way that albino rats administered with a high-protein diet, react to methanolic root extracts of *Lagerstroemia speciosa* anticalciuric properties. Medicinal plants are frequently utilised in folk medicine to treat a wide range of illnesses. Banaba is the common name for *L. speciosa*, a member of the Lythraceae family; Poomaruthu is its Tamil name. The test animals were given a diet high in protein and low in protein as well as 45 days of treatment with methanolic root extracts of *L. speciosa*. The experiment was scheduled with a sample collection, biochemical inquiries, and histopathological exams. The experiment had been scheduled with a sample collection, biochemical investigations, and histopathological evaluations. The biochemical data reveal that as compared to the control, III, and V groups, the low-protein diet groups II and IV had greater blood urea concentrations. The low-protein diet groups II and IV exhibited an increased blood creatinine concentration in contrast to the control, III, and V groups, while group V (H.P+MRE 500 mg/kg) demonstrated a nearly identical outcome to that of the control groups. *L. speciosa* root extracts in methanol are given to groups to control creatinine concentration. The normal control group's histopathological analysis revealed tubules with a single epithelial lining along the outermost layer that were normal in size.

Keywords: *Lagerstroemia speciosa*, Calciuria, Blood urea, Serum creatinine, Kidney disorder, high-protein diet, low-protein diet.

1. INTRODUCTION
Kidney stones are becoming more common, a large number of people are suffering from urinary stones problems all over the globe [1]. The formation of kidney stones is influenced by lifestyle; food habits or dietary habit, sometimes may be intake of proteins. The kidney stone composition is of calcium oxalate or calcium phosphate [2]. It can also be measured in terms of the amount per mass of creatinine, which is helpful for estimating the urinary calcium excretion in a spot urine sample because urinary creatinine clearance is largely unaffected by variations in free water clearance that, for instance, occur in dehydration and would cloud the interpretation of the urinary calcium in a spot urine sample.

Proteins must be consumed by the human body through nutrition [3]. They're one of the ingredients of body tissue and can be eaten as fuel. The most crucial factor and distinctive feature of proteins from a nutritional standpoint is their amino acid makeup. Peptide bonds bind the amino acid polymer chains that compose proteins. During human digestion, hydrochloric acid and proteins known as proteases break down proteins into smaller polypeptide chains in the stomach. According to Genton *et al*. [4], this is required for the absorption of the essential amino acids that the body is unable to biosynthesize.

Calcinuria refers to the level of calcium in the urine. The typical level of urine calcium in a urinalysis can be calculated in units per hour (typically per 24 hours). An average adult typically excretes 100-250 mg of calcium in urine over the course of a day. An abnormally high amount of urinary calcium is called Hypercalciuria and an abnormally low amount is called Hypocalciuric [5]. In

concentrated urine, minerals and acid salts produce hard deposits called kidney stones that attach to other deposits. Despite the fact that they frequently do not result in long-term damage, they can be uncomfortable when travelling through the urinary tract. Severe discomfort, usually on one side of the abdomen that is frequently accompanied by nausea is the most prevalent symptom.

Many people from India worked on medicinal plants used for kidney pain [6]. WHO identified 20,000 medicinal plants overall on our planet. *Lagerstroemia speciosa,* which is commonly called Banaba or crape myrtle in the Philippines, belongs to the family Lythraceae. In India, it is commonly called the Queen's flower and the Pride of India. The roots were used as astringents, stimulants, and febrifuges; they were also used for stomach problems [7]. The study investigates the potential explanations for the methanolic root extract of *L. speciosa's* nephroprotective effect and its anti - calciuria activity against protein diet ingested in albino rats.

2. MATERIAL AND METHODS

2.1. Collection and maintenance of the experimental animals

Healthy untreated Albino male rats weighing 120-180 g were used in all experiments, and rats weighing between 120 and 180g were used for the acute toxic study. All animals were collected from the animal house, KMCH, College of Pharmacy, Coimbatore. The animals were grouped and housed in polyacrylic cages and provided pellets and tap water ad libitum. All mice were maintained under specified pathogen-free conditions in a barrier facility and under a strict 12-hour light cycle. (Approval No: KMCRET/ RERC/ Ph.D. /23/ 2021).

2.2. Collection and identification of plant materials

The plant *L. speciosa* was collected from the Government Arts College Girls Hostel in Coimbatore, Tamil Nadu, India. The authenticity of the plant was confirmed by Dr. M. U. Sharief, scientist 'E' and head of office, Botanical Survey of India, Southern Circle, Coimbatore, by referring to the deposited specimen. The voucher number for the specimen is BSI/SRC/5/23/2021/ Tech/236.

Phytochemical screening for plant constituents was done using the method described by Sofowora [8], Trease, and Evans [9], which shows that a number of both primary and secondary metabolites are present in the methanolic leaf extract of *L. speciosa*. Secondary metabolites that were found to be present were alkaloids, cardiac glycosides, flavonoids, phenols, reducing sugar, saponins, steroids, tannins, phytosterols, amino acids, and proteins [10]. Several of these constituents may possibly be responsible for the plant's hepatoprotective activity. The GC-MS examination of the *L. speciosa* leaf extract was performed by "The South Indian Textile Research Association, CBE-14". (Voucher No. E2100701).

2.3. Preparation of *Lagerstroemia speciosa* Root Extract

The fresh roots of this species were collected, washed under running tap water, followed by sterile distilled water [11], cut into small pieces, and then shade dried for several days. The pieces were then ground into coarse powder by using a high-capacity grinding machine, passed through a sieve, and stored in a tight container. The powdered plant samples (100 g/1000 mL) were taken in a clean, flat-bottomed glass container and soaked in methanol for three days. The whole mixture then underwent coarse filtration by a piece of clean, white cotton material. The solvent of the extract was reduced to room temperature and stored for further use.

2.4. Phytochemical Analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the methanolic extracts of the root parts of *L. speciosa.* The dried plant extract was dissolved in a methanol solution for phytochemical analysis.

2.5. Drug preparation

The sample was reconstituted using distilled water to prepare a 6 mg/mL w/v concentration of the compound.

2.6. Experimental Design

The effect of *Lagerstroemia speciosa* methanolic root extract on low- and high-protein rats was studied. All the rats received treatment for 45 days and were randomly distributed into five groups of six animals each.

Group I : Animals received normal saline (1 mL/100 g BW/day) for 45 days.

Group II : Animals received low-dose protein (250 mg/kg BW/day) for 45 days.

Group III : Animals received high-dose protein (500 mg/kg BW/day) for 45 days.

Group IV : Animals received a low dose of protein (250 mg/kg) and a low dose of Methanolic Root Extract. For 45 days, drink 250 ml/Kg BW per day.

Group V : Animals received high protein (500 mg/kg) and a high dose of Methanolic Root Extract for 45 days, drink 500 ml/Kg BW per day.

2.7. Acute oral toxicity study

Immediately after dosing, the rats were observed continuously for the first 6 hours for any toxicity signs such as gross changes in the skin, fur, eyes, mucous membranes, or circulatory, respiratory, or behavioral patterns. They were then kept under observation for 5 days after drug administration to find out the mortality rate, if any. Observations were made twice daily. No deaths occurred during the acute oral toxicity study.

2.8. Animal Sacrifice and collection of samples

The animals were sacrificed by decapitation. Rat blood was collected by retro-orbital plexus puncture and centrifuged at 3000 rpm for 5 min, and serum samples were separated and stored at -80°C.

Rat kidneys were removed, cleaned with ice-cold saline, weighed, and chilled on crushed ice. A piece of the liver was fixed in 10% buffered formalin for subsequent morphologic analysis.

Another part was homogenized in a 10-fold volume of ice-cold sodium and potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl. The homogenates were centrifuged at 600 g at 4°C for 10 min. The supernatant, referred to as homogenate, was stored at -80°C until used.

2.9. Biochemical studies

The blood samples were collected through the medial cantus into EDTA bottles for biochemical studies. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 minutes and analyzed for various biochemical parameters.

2.10. Histopathological studies

Both kidneys were removed aseptically and inspected for evidence of cholesterol. The organ was dissected into two halves and incubated in 10% formalin overnight at 4°C.

The tissues of the organ were embedded in 2% agar for paraffin processing. The sacrificed organ was excised, rinsed in ice-cold normal saline, followed by 10% formalin fixed for samples. Paraffin sections of buffered formalin-fixed kidney samples were stained with hematoxylin-eosin to study the histological structure of the control and treated rat livers [12].

2.11. Statistical analysis

The data was descriptively analyzed using one-way analysis of variance (ANOVA).

3. RESULTS

3.1. Phytochemical analysis

The phytochemical study revealed that the crude methanolic root extract of *L.speciosa* contained steroids, amino acids, alkaloids, phenols, tannins, flavonoids, phytosterols, cardiac glycosides, saponins, and reducing sugars. (Table No. 1)

Table 1: Phytochemical analysis of methanolic root extracts of *Lagerstroemia speciosa*

Phytocomponents	Methanolic Root Extract
Steroids	++
Amino acids	++
Alkaloids	++
Phenols	+++
Tannins	++
Flavonoids	+++
Phytosterols	++
Cardiac glycosides	+++
Saponins	+++
Reducing sugars	++

'++' indicates the phytoconstituents present in a moderate level
'+++' indicates the phytoconstituents present abundantly

3.2. GC-MS analysis

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides, and food additives. *Lagerstroemia speciosa* is native to tropical and subtropical regions of Asia. The GC-MS investigation of *Lagerstroemia speciosa* root revealed the presence of the following chemical constituents in a major ratio with several other chemical constituents: 1H-Pyrrole-2-carboxaldehyde, 1-methyl-propargyl ether, 6-Ethyl-5,6-dihydro-2H-pyran-2-one, Methane, chlorofluoro-, 1,2,4,5-Tetrazin-3-amine, 6-methyl-, 2-(4-Methyl-1H-1,2,3-triazol-1-yl)ethan-1-amine, 1-Methylpyrazol-4-amine, Ethyl bromide, 1H-Pyrrole-2-carboxaldehyde, 1-methyl-, 2-Butyn-1-al diethyl acetal, 2,3-Hexanedione, Nitrogen trifluoride, Heptyl methyl ethylphosphonate, 1H-Pyrrole-2-carboxaldehyde, etc. (Table 2).

3.3. General and physical observation

All the animals showed normal appearance and appetite. However, increased water intake was observed to go

hand in hand with an increase in the dosage of extract administration across the groups. Increased urinary and faecal output was observed in group III and IV animals administered 500 mg/kg of the extract in comparison to other groups. At the end of the experiment, seemingly dose-dependent, significant increases in body weight were also observed in all the groups of the experimental animals.

3.4. Acute toxicity

The crude methanol root extract is non-toxic in rats; it was well tolerated at concentrations of 250 and 500 ml/kg. No biochemical or histological changes were recorded in the acute toxicity study. No mortality or toxic reaction was recorded in rats after administration of the methanolic crude extract of *Lagerstroemia speciosa* roots (250 and 500 mg/kg, orally).

3.5. Biochemical analysis

3.5.1. Blood urea nitrogen

Administration of a methanolic extract of *L.speciosa* for 45 days with 250 mg/kg and 500 mg/kg in groups I and II showed a significant change in the serum levels of blood urea nitrogen concentrations compared with the only protein diet groups. Low-protein diet groups II and IV showed higher blood urea concentrations compared with the control, III, and V groups. Group V (H.P. +MRE 500 mg/kg) showed an almost similar result to that of the Group I control (Table 4).

Table 2: Important compounds identified in the GC-MS analysis of root extract of *lagerstroemia speciosa*

S. No	Compound Name	Molecular Formula	Match Factor
1	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	C6H7NO	93.7
2	Methyl propargyl ether	C4H6O	93.5
3	6-Ethyl-5,6-dihydro-2H-pyran-2-one	C7H10O2	89.9
4	Methane, chlorofluoro-	CH2ClF	87.2
5	1,2,4,5-Tetrazin-3-amine, 6-methyl-	C3H5N5	86.9
6	2-(4-Methyl-1H-1,2,3-triazol-1-yl)ethan-1-amine	C5H10N4	86.1
7	1-Methylpyrazol-4-amine	C4H7N3	86.0
8	Ethyl bromide	C2H5Br	85.9
9	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	C6H7NO	85.4
10	2-Butyn-1-al diethyl acetyl	C8H14O2	85.3
11	2,3-Hexanedione	C6H10O2	84.8
12	Nitrogen trifluoride	F3N	84.7
13	Heptyl methyl ethylphosphonate	C10H23O3P	84.2
14	1H-Pyrrole-2-carboxaldehyde	C5H5NO	83.6

Table 3: Initial and final body weight of experimental groups

Group	Control	L.P 250mg/kg	H.P 500mg/kg	L.P+MRE 250mg/kg	H.P 500mg/kg
Initial Body Weight	131±2.46	126±3.2	128±1.52	132±2.2.2	130±2.14
FinalBody Weight	173±2.82	187±11	190±10.6	141±46.5	150±30.2

The values are expressed as mean±SEM.

Table 4: Biochemical analysis (blood urea nitrogen and serum creatinine)

Group	Control	L.P 250mg/kg	H.P 500mg/kg	L.P+MRE 250mg/kg	H.P 500mg/kg
Urea (mg/dl)	27.7±0.808	64±1.755	37.1±1.101	51.9±1.545	27.8±0.935
Creatinine (mg/dl)	0.767±0.203	2.43±0.606	1.83±0.318	2.1±0.808	1.43±0.437

The values are expressed as mean±SEM.

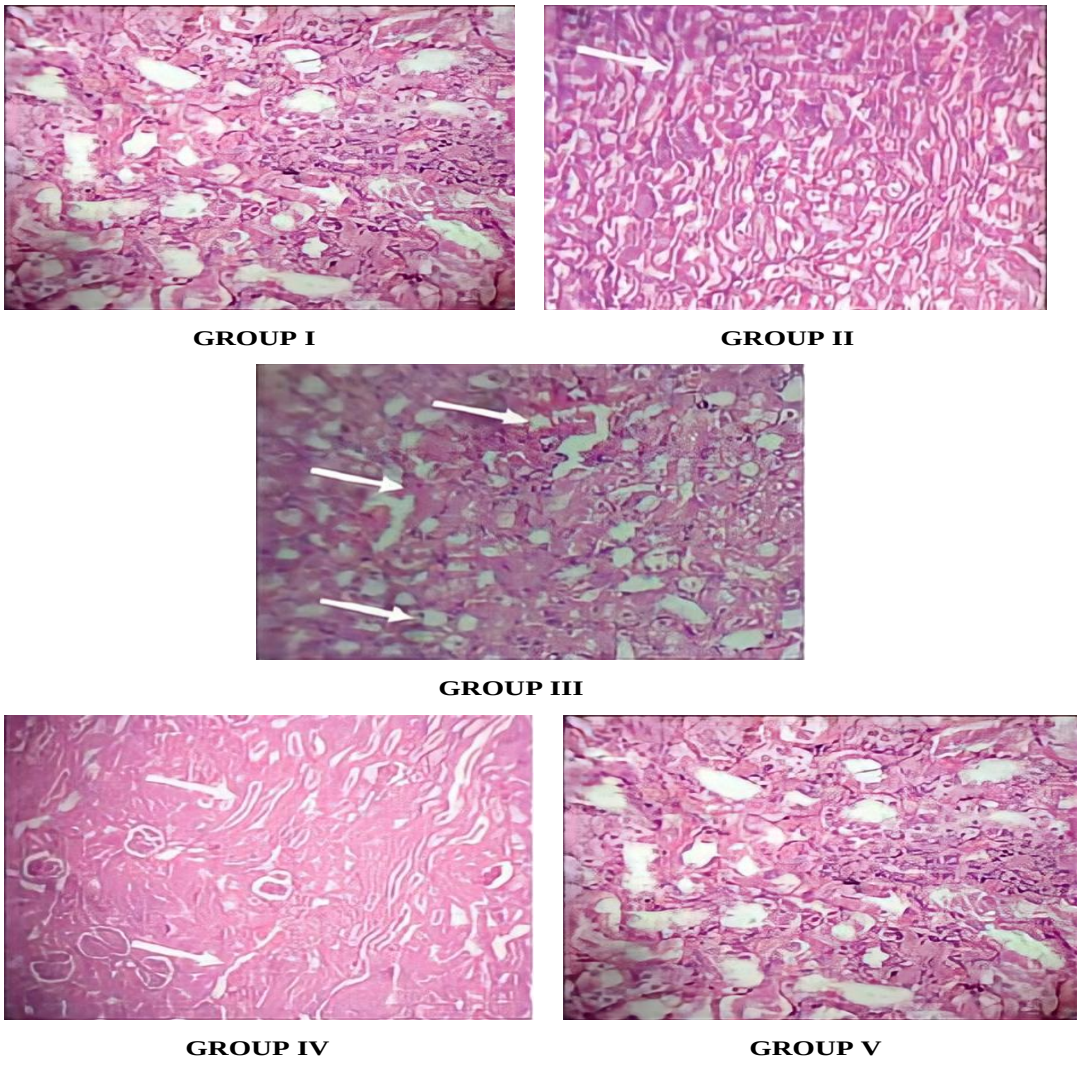
3.5.2. Serum creatinine

Serum creatinine concentration of the blood was examined. Low-protein diet groups II and IV showed higher serum creatinine concentrations compared with the control, III, and V groups. Creatinine concentration is controlled in groups administered with methanolic root extracts of *L. speciosa* (Table 4).

3.6. Histopathological analysis

Microscopic examination using polarized light of kidney sections showed intratubular and interstitial crystal deposits in Group II and III rats. However, rats treated with a methanolic extract of *L. speciosa* had far less kidney calcification. Histopathological examination of the normal control group showed normal-sized tubules

with a single epithelial lining along the margin. In the group II and III rats, there was marked dilatation of the tubules and total degeneration of the epithelial lining with infiltration of the inflammatory cells into the interstitial space. In Groups IV and V, the specimen showed characteristics similar to normal control Group I with regenerative cells (Fig. 2).



Group I *- section showed normal size tubules with single epithelial lining along the margin;* ***Group II and Group III*** *- sections showed intratubular and interstitial crystal deposits, marked dilatation of the tubules and total degeneration of the epithelial lining with infiltration of the inflammatory cells into the interstitial space;* ***Group IV and Group V*** *- sections showed characters similar to normal control group with regenerative cells.*

Fig. 1: Histopathological Analysis of the Organ Kidney (H & E staining)

3.7. Statistical analysis

All values were expressed as the mean ± SEM. Differences between groups were evaluated by one-way ANOVA.

Body Weight: Statistical Comparison: In each group (n = 6), each value represents the mean ± SEM. A one-way ANOVA, followed by a Dunnett comparison, was performed. Control group was compared with group II. ***(***P<0.001-**P<0.01, *P<0.05)*** treated groups III, IV, and V were compared with group I (Table 3).

Urea and Creatinine: Statistical Comparison: In each group (n = 6), each value represents the mean ± SEM. A one-way ANOVA, followed by a Dunnett comparison, was performed. Control group was compared with group II. ***(***P<0.001-**P<0.01, *P<0.05)*** treated groups III, IV, and V were compared with group I (Table 4).

4. DISCUSSION

Proteins are crucial for the human body's tissue and fuel, broken down by proteases in the stomach. The kidney's function is crucial for maintaining body homeostasis by excreting metabolic waste and regulating fluid volume, electrolyte composition, and acid-base balance [13]. This study analyzes body weight, serum concentrations, and kidney histopathology to evaluate renal function, with creatinine and urea being markers of renal function. Calciuria decreases glomerular filtration due to urinary system stones and renal parenchyma damage, causing waste products like urea, creatinine, and uric acid accumulation.

Traditional medicine uses these secondary metabolites for anti-inflammatory, anti-analgesic, and anti-diuretic properties. Phytochemicals like phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, and phytosterols have medicinal and physiological activities.

L. speciosa's anticalcineurin effect decreases urea and creatinine levels in MRE-ingested groups.The extract of *L. speciosa* significantly decreased urea and creatinine levels in IV and V groups, suggesting recovery of renal function. *L. speciosa's* methanolic extract may prevent stone formation and contribute to its anticalcineurin properties, possibly due to its antibacterial activity and active principles like flavonoids, saponins, and polyphenols.

5. CONCLUSION

Medicinal plants play a major part in maintaining human health and preventing illnesses, such as kidney disease. However, there is now insufficient pharmacological data to support the use of these plants and their phytonutrients in the prevention of renal disorders. In this work, we provide data regarding the phytochemicals and pharmacological mechanisms of *Lagerstroemia speciosa* plants for the management or prevention of calcinuria. The *L.speciosa* extract utilized in this investigation revealed Based on the restoration of biochemical parameters like body weight, blood urea, and serum creatinine content against various types of free radicals as demonstrated during the experimental study, nephroprotective potential in comparison to a normal protein diet, it was assumed that the mechanism of nephro-protection was primarily due to antioxidants.

Conflict of interest

None declared

Source of funding

None declared

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