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Studies on Diuretic Effect of *Lagerstroemia Speciosa* Linn. Leaf Extracts in Normal Rats

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ABSTRACT

Lagerstroemia speciosa L is a medicinal, ornamental, decidous small tree native of China, commonly cultivated in gardens through out India for beautiful flowers. The leaves of this tropical plant have been used as a folk medicine for treatment of diabetes and kidney diseases. Many pharmacological studies has been carried out in *L. speciosa* but the diuretic activity of the plant has not been studied yet. Many herbal diuretics exert their action by directly effecting electrolyte balance of minerals. The ethyl acetate, ethanol, methanol and water extract of *Lagerstroemia speciosa* L was evaluated for diuretic activity. Diuretic effect was carried out in rats by measuring the urine volume by 1, 2, 4, 6 hours and later at 24 hours. Positive controls (furosemide and mannitol (20mg/kg and 100mg/kg) were given intraperitoneal and intravenous route respectively. The extracts were administered orally at the dose of 250mg/kg b.wt. Na^+, K^+ and Cl^- concentrations and urine volumes were determined. Na^+/ K^+ ratio was higher in aqueous extract and followed by ethanol, ethylacetate and methanol extracts. The aqueous extracts show best diuretic effect when compared with other extracts. It can be concluded that all the extracts showed diuretic effect and cation excretion outstandingly.

Keywords: Furosemide, Mannitol, electrolytes, proximal tubule, *L.speciosa*

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INTRODUCTION

Lagerstroemia speciosa Linn called as banaba, is a tropical plant found in many parts of Southeast Asia including the Philippines, Vietnam, Malaysia, India and southern China. The leaves and other parts of banaba are used widely by the Philippines, Taiwan, China and Japan as a tea preparation. This tea is consumed as a natural means for a variety of reasons involving the kidneys, such as dissolving kidney stones, kidney cleanses, and kidney health in general. *L. speciosa* has become relatively popular in the form of health-promoting tea products in Eastern Asia and the United States [1]. The leaves of the plants are used in the treatment of diabetes [2] and also the tribal people for heart diseases use it. It is also used for abdominal pains, mouth ulcers, stimulant and febrifuge [3].

The subject of phytochemistry has been developed in recent years as a distinct discipline somewhere in between natural product organic chemistry and plant biochemistry and it is closely related to both. It is concerned with enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their metabolism, their natural distribution and their biological function.

In earlier studies the extract of this plant is reported to have an antioxidant, anti inflammatory, hepatoprotective, nephroprotective agent [4,5] and anti-diabetic properties. A number of pharmacological properties have been reported where the diuretic activity has not been experimentally proved. The present investigation was undertaken to confirm the diuretic activity of different extracts of *L. speciosa* leaves. The diuretics are drugs that act on the kidney and are able to increase the volume of urine excreted, the reason why are used in cardiac failure, chronic and moderate cardiac insufficiencies, acute oedema of the lung, nephritic edema syndrome, arterial hypertension, diseases related with the retention of fluids etc [6,7]. In the present study we have used fursemide and mannitol as reference standards. Furosemide is a sulphonamyl derivative which is a high efficacy diuretic which has its primary action on medullary ascending limb of loop of henle and can produce substantial effect because of limited capacity for salt absorption in distal tubule and collecting duct. Mannitol, which is a sugar, is an osmotic diuretic, when administered intravenously, is not metabolized and rapidly filtered by glomeruli but not reabsorbed. It causes water to be retained in the proximal tubule and descending limb of henle.

MATERIALS AND METHODS

Plant material

The leaves of *Lagerstroemia speciosa* Linn were collected from Amala Ayurvedic Hospital premises, Trichur, Kerala, India. Dr. C.N. Sunil, Department of Botany, S.N.M College, Maliankara, Kerala, authenticated the plant materials and a voucher specimen (BSI No. 62373) was kept at Fr. Gabriel Herbarium, Amala Ayurvedic Hospital, Trichur.

Preparation of plant extract

Shade dried leaves were powdered, subjected to successive soxhlet extraction using a series of solvents of increasing polarity starting from petroleum ether (for defatting), ethyl acetate, ethanol, methanol and water respectively. The solvents were concentrated separately by vacuum evaporator to get the residue. The extracts were further dried in desiccators and the yields of the extracts were 3.56, 4, 14.2, 3.19 and 17.47% respectively.

Animals

Male Wistar rats (175-200g) Male Balb/C mice (25-30g) were used for the experiments. They were housed in environmental conditions and fed with standard rodent diet and water *ad libitum.* All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC) and followed the guidelines of IAEC.

Phytochemical analysis

Phytochemical analysis of the major phytoconstituents of the plant extracts were undertaken using standard qualitative colour tests using the conventional protocols [8].

Determination of macro elements using flame photometry

Preparation of sodium, potassium and calcium standard solution- Analar quantity of NaCl and KCl is accurately weighed and dissolved it in exactly 250 mL of double distilled water. It is diluted (1:100) to get 1mg Na/100 mL and 1mg K/100 mL, which is equivalent to 10 ppm. Analar quantity of $CaCO_3$ is accurately weighed and is dissolved in minimum quantity 1:1 HCl and it is made up exactly to 250 mL with double distilled water and it is diluted to get 10 mg Ca/100 mL which is equivalent to 100ppm.

Preparation of test solutions-An accurately weighed amount of ash of the plant materials was digested with 5 mL of 10% HCl. This was filtered through Whatman No. 4 filter paper and the residue was washed with hot water, cooled and made to volume. The sample solutions were then compared in the flame photometer against standard solutions of NaCl, KCl, and $CaCO_3$ containing the same amount of HCl. The concentrations of the sodium, potassium and calcium ions were collected by extrapolation method.

Determination of heavy metals (Atomic Absorption Spectrophotometer - AAS)

Heavy metals include the elements arsenic, cadmium, mercury and lead were detected at our lab using atomic absorption spectrometer (AAS), (Thermoelectron, UK) 'S' series model with VP100 vapour generator [9].

Preparation of Sample solutions

The leaves of *L. speciosa* were cleaned visually and dried at 150^{o}c to a constant weight. The dried materials were then grounded to a fine powder and were used for dry ashing. Pre-cleaned silica crucibles were heated to 600^{o} C till the weight of the crucibles was constant. The determination of mercury, lead, arsenic and cadmium was conducted by Atomic Absorption Spectrophotometry (AAS). Standard solutions of all the metal elements were prepared as per the standard procedures reported in the operating manual of the instrument and standard curves were prepared for the same [10].

Assessment of diuretic activity

Male Wistar rats (175-200g) were purchased from Small Animals Breeding Station, Mannuthy, Thrissur, Kerala. They were maintained under standard conditions of temperature and humidity. The method of Lipschitz et *al* [11] was employed for the assessment of diuretic activity. The dose of the extract used in the present study was based on our toxicity studies reported earlier [5]. Six groups of six rats each were fasted and deprived of water for eighteen hours prior to the experiment. On the day of experiment, normal group of animals were given normal saline orally (25 ml/kg body weight.) and the treated groups were given 250mg/kg bodyweight of extracts of ethyl acetate, ethanol and water. The standard groups were given furosemide (20mg/kg) intraperitoneally and mannitol (100mg/kg) intravenously**.** The rats were placed in metabolic cages specially designed to separate feacal matter and urine. The urine volume was registered at 1, 2, 4, 6 and 24 hours post administration. During this period no food or water was given to the animals. The total urine volume was measured for both control and treated animals. The sodium, potassium and chloride ion concentration in the urine samples were determined.

STATISTICAL ANALYSIS

All data were analyzed through one way analysis of variance (ANOVA) followed by Multiple Comparison Range Test (means and 95.0 Percent Tukey HSD). The difference between the test groups and control was determined by least significant difference method at $p<0.05$ confidence levels.

RESULTS

Determination of qualitative phytochemical analysis

The chemical test showed that *L. speciosa* contains saponins in ethanol, methanol and water extracts. Tannins are reported in all extracts of the plant. But alkaloids showed positive in EtOH and MeOH extract of *L. speciosa* and except water extract, flavonoids are presented in all other extracts of *L. speciosa*.

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TABLE 1: Qualitative phytochemical evaluations of the extracts *L. speciosa*

Constituents	Observations			
	Ethyl acetate (EtOAc)	Ethanol (EtOH)	Methanol (MeOH)	Water (Aqueous)
Saponins	-	+	+	+
Tannins	+	+	+	+
Alkaloids	-	+	+	-
Sterols	+	-	+	+
Glycosides	-	+	-	-
Flavonoids	+	+	+	-

Determination of calcium, potassium and sodium levels of *L. speciosa*

The amount of macronutrients present in the plant parts were measured using flame photometry. The amount of calcium, potassium and sodium present in the leaves of *L. speciosa* were found to be 143.85, 85.1 and 12.3 µg/g respectively (data not shown).

Estimation of heavy metal content of *L. speciosa*

The heavy metal content was estimated in the plant parts after complete digition and estimated by AAS and levels were compared with the WHO standard and tabulated (Table 2). The mercury content was found to be 62.6 PPB in the leaves of *L. speciosa*. It was found that the *L. speciosa* contains 2.02, 1.16 and 0.26 PPM of arsenic, lead and cadmium respectively. The result showed that none of the heavy metals presented in the plant parts are not above the WHO recommended level.

TABLE 2 Data showing the Heavy metal content of *L. speciosa*

Heavy metals	*L. speciosa*	Recommended level (WHO)
Mercury	0.626 PPM	1 PPM
Arsenic	2.02 PPM	3 PPM
Lead	1.16 PPM	10 PPM
Cadmium	0.26 PPM	0.3 PPM

Determination of diuretic activity:

It was found that the ethyl acetate, ethanol, methanol and aqueous extract showed diuretic activity when compared with the standard furosemide and mannitol (Figure 1). In the normal rats the diuresis began passed one hour of the administration, showing low volumes of urine excreted until completing 43.2 mL at 24 hours. The group dealt with furosemide (positive

control), the beginning of the diuretic action was at 60 minutes. A final volume of 76.4 mL was reached being significantly different from the obtained in the negative control group ($p< 0.05$). In group III dealing with mannitol, does not show significant increase in urine volume. The beginning of urine for the watery extract of the *L. speciosa* was also at 60 minutes post administration, but the volume was smaller (12.5 mL), differing significantly from the values obtained with furosemide ($p<0.001$) being reached a total volume of 29.4 mL. The order of activity of increase of urine output was slightly greater for aqueous extract (12.5 mL) than that of ethanol extract (12.1 mL) at the end of first hour but the ethyl acetate and methanol showed lesser effect after 60 minutes. But at the end of fourth hour the methanol extract showed better activity that urine output was increased to 29.7 mL which is comparable to ethanol and aqueous extract at the end of fourth hour, showing that the methanol extract is showing more activity after this time interval. But the increase in the urine volume of ethanol extract after four hours up to six hours was found to be very less when compared with all the other extracts. The electrolytes, Na^+, K^+ and Cl^- levels were significantly ($P<0.001$) high in standard drug treatment when compared with normal group. The plant extracts increased the electrolytes level in urine and it is not significant. The EtOH extract showed grater electrolytes level than the EtOAc extracts. Similarly it was obtained an increase of the excretion of Na^+ in the urine was significantly superior to the one registered in the negative control group ($p<0.001$) and very highly significantly superior compared with furosemide group (Table 3).

TABLE 3 : Effect of extracts of *L. speciosa* on electrolyte level of Wistar rats

Group	Dose mg/kg	Electrolyte concentration in m eq/L			
		Na^+	K^+	Cl^-	Na^+/K^+
Normal	Saline 5ml/kg	178.25 ± 12.8^{a}	87.20 ± 10.5^{a}	113.21 ± 8.9^{a}	2.04
Furosemide	20mg/kg	323.61 ± 17.4^{b*}	123.56 ± 10.8^{b*}	215.70 ± 11.4^{b*}	2.61
Mannitol	100mg/kg	261.53 ± 10.5^{c*†}	112.32 ± 6.2^{bc*}	123.62 ± 9.1^{ac†}	2.32
L. speciosa EtOAc extract	250mg/kg	149.16 ± 9.9^{ad†}	83.15 ± 6.4^{ad†}	118.81 ± 6.2^{acd†}	1.79
L. speciosa EtOH extract	250 mg/kg	186.11 ± 12.5^{ae†‡}	99.54 ± 8.5^{acde†}	156.12 ± 7.7^{ae†‡}	1.87
L. speciosa MeOH extract	250mg/kg	192.64 ± 8.0^{af†}	109.14 ± 11.1^{acf†}	134.81 ± 12.5^{acf†}	1.77
L. speciosa Aq extract	250 mg/kg	243.15 ± 11.5^{afg†}	121.17 ± 10.14^{abcf}	165.23 ± 7.2^{ag†‡}	2.01

Values are mean ± S.D, n=6 animals

*P<0.001, Comparison between normal group with other groups

† P<0.001, Comparison between control group with other groups

‡ P<0.01, Comparison between different solvent extracts.

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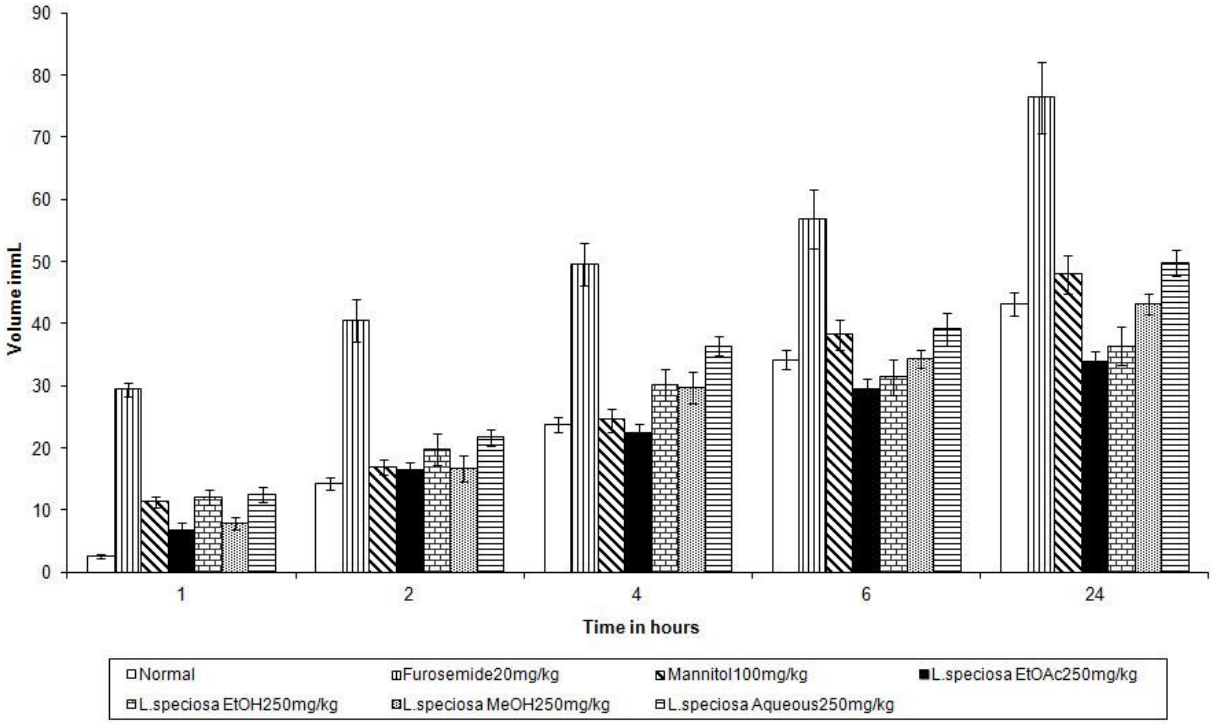

The K^+ concentration in urine, was very high significantly superior compared with negative control, furosemide and mannitol groups ($p<0.001$). The aqueous extract administration increases the Na^+ concentration than other extract treated group. The ethyl acetate fraction showed lesser urinary excretion when compared with other extracts. Increased ratio of Na^+/K^+ represents the potent activity of a drug. The standard drugs showed elevated level when compared with normal group. The Na^+/ K^+ ratio of the aqueous extract treated group showed higher level than other extracts treated groups.

DISCUSSION

Kidney, the excretory organ of our body serves the important function of excretion of waste products, regulation of fluid volume and electrolyte content of the extracellular fluid. Diuretics are drugs capable of increasing levels of urine. In the normal rats diuresis began with low volumes of urine excreted until completing 24 hours. The level of excreted Na^+ and K^+ in urine was equally low. The furosemide (positive control) treated group, the diuretic action start at 60 minutes and increased significantly ($p< 0.05$) from normal rats. In the mannitol administered group showed lesser urine volume when compared with furosemide. The beginning of urine for the watery extract of the *L. speciosa* was also at 60 minutes post administration, but the volume was smaller than furosemide.

The presence of phytoconstituents like terpenoids, saponins, flavonoids has been reported previously to be responsible for the diuretic activity in plants [12,13]. The maximum volume of urine at the end of 24 hours was for EtOH extract may be due to the presence of flavonoids, saponins, tannins [14] etc. The best diuretic effects could be associated to the

flavonoid content, also it promote high levels of Na^+ and K^+ in urine. There are correspondences between the volume of urine and the concentration of Na^+, this aspect is logical because the mechanism of action of diuretic drugs is to decrease the tubular reabsorption of this ion, it produces the dragging of the osmotic equivalent of water, other explanation that can support this, is the high ion concentrations in this medicinal plants. [15, 16].

Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They increase plasma volume and subsequently venous return to the heart. This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus diuretics play an important role in hypertensive patients. The electrolytes, Na^+, K^+ and Cl^- levels were significantly ($P<0.001$) high in standard drug treatment when compared with normal group. The plant extracts increased the electrolytes level in urine and it is not significant. Increased ratio of Na^+/K^+ represents the potent activity of a drug. The standard drugs showed elevated level when compared with normal group.

The ethyl acetate fraction did not increase urinary excretion when compared with other extracts. All extracts did not increase the Na^+ concentration when compared with the positive controls. It was reported earlier that 30 to 70% of K^+ filtered by the glomerulus is reabsorbed by the proximal convoluted tubule [17] by a combination of three processes: active transport, paracellular diffusion and solvent drag [18]. The mechanism of action are complex and involve a variety of energy dependent trans membrane pumps as well as channels in between the loose fitting cells of the proximal tubule (PT). About 80% of the nephrons lie in outer cortex, having short loops of Henle and low Na^+ reabsorptive capacity where as 20% are juxta medullary possessing long loops of Henle and are responsible for creating the cortico medullary osmotic gradient. The redistribution of blood flow between these two types of nephrons can alter salt and water excretion. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect [19]. The chloride ion excretion was not elevated significantly when compared with the normal animals and the results are indicating that the extract is a potent natriuretic [20].

CONCLUSION

The extracts showed diuretic effects after the administration of 250mg/kg body weight dose. Out of these extracts water extract showed better diuretic properties and also superior urine excretions of of Na^+ and K^+. Further studies like isolation and characterization of diuretic principle from the plant is needed to understand and confirm the exact mechanism of action.

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