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A Prospective study of Anti-Diabetic activity of *Lagerstroemia speciosa* Linn.

Merlin Jayalal .L .P†

Abstract

Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases. Diabetes mellitus is one of the leading cause of death in many developed countries. The incidence of diabetes is increasing at an alarming rate in India. It was estimated that India which had 19.4 million diabetes in 1995 is expected to register a near threefold increase by. Many plants reported to be useful for the treatment of diabetes mellitus in ayurvedic medicine, are being tested for their hypoglycemic activity in experimental animals *Lagerstroemia flos- reginae* is one such plant commonly found as shade trees in Kerala. In Ayurveda both root and leaves are used in the treatment of diabetes. The main objective of this study was to assess the antidiabetic effect of the alcohol extracted leaves of *Lagerstroemia flos- reginae* in alloxan induced diabetic rats in terms of controlling blood glucose level, lipid profile, bilirubin, uric acid in serum and lipid peroxides and glutathione in the liver of the experimental animals. The present study has been undertaken to observe the protective effect of the active constituents of *Lagerstroemia flos- reginae* leaf extracts against alloxan induced diabetes in experimental animal model. The activity of the active constituents was compared with Daonil –a standard drug.

Key words : Diabetes Mellitus, *Lagerstroemia flos- reginae*

1. Introduction

Herbal technology circumscribes all the advancing technical frontiers (except genes) meant to tap myriads of modes of manipulating plants around us. A large number of technologies have been developed to harvest the bountiful products that the plant manufacture, including Natural Dyes, Biofertilizers, Biopesticides, Biofuel, Herbal Medicines. Search for novel anti-diabetic drugs from natural sources has intensified[1] a number of herbal preparations and plant extracts has been found to use with varying degree of success in the management of diabetes[2-4]. Life being synthetic, people are turning towards nature for products available from various natural sources-plants, animals and micro-organism. Diabetes Mellitus occurs when pancreas do not produce enough insulin, a hormone that helps our body to use sugar (glucose) to yield energy[5]. Diabetes is a group of disease in which the regulatory activity of insulin may be defective in different ways. Deficiency of trace elements such as chromium, copper and zinc play a role in the pathogenesis[6]. Excessive consumption of alcohol induce hyperglycemia and diabetes mellitus in patients.

In Ayurveda diabetes mellitus is known as “Madhumeha”, in sense “honey in urine”. According to Charaka, the father of ayurveda, diabetes mellitus is a disease where the body constituents are melted in to sugar[7]. Many herbal medicines in different oral formulation were recommended for madhumeha during the time of Charaka and Susrutha. Crude drugs extracted from plant source such as garlic *Allium sativum,* neem *Azadirachta indica* and meshasingi *Gymnema sylvestre* possess hypoglycemic activity in experimental animals[8].

Lagerstroemia speciosa is found in Batan islands and northern Luzon to palawan, Mindanao and the sili Archipelago is most or all island and provinces, chief in secondary forest at low and medium attitudes.

Lagerstroemia genus have about 50 species. *Lagerstroemia flos- reginae* is one of the most important species, that possess immense therapeutic values. It is a dicotyledonous tree, having awesome flowers and grows about 15 feet and found mostly in southern parts

Department of Biochemistry, Bharathidasan college of Arts and Science, Ellispettai, Erode-638116, Tamilnadu, India

†Corresponding author : lpmjlal@gmail.com
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of India as shading trees along the national highways and most commonly called as "Pride of the forest". The old leaves and ripe fruits are the parts of *Lagerstroemia flos- reginae* that contain the greatest amount of Insulin like principle. Twenty grams of old leaves or fruits, dried for one to two weeks in 100 cc, 20 percent decoction were found to have the activity equivalent to form 6 to 7.7 units of insulin in lowering blood sugar. The young leaves and flowers have an activity that range from 4.4 to 5.4 units of insulin per 100 cc of 20 percent decoction or found to have the activity equivalent to form 6 to 7.7 units of insulin in lowering blood sugar. The mature leaves, young leaves, and flowers have an activity that range from 4.4 to 5.4 units of insulin per 100 cc of 20 percent decoction or equivalent to around 70 percent of the activity of the leaves or fruit. The wood does not contain the insulin – like principle while the bark and roots contain a very small amount. The Insulin like principle deteriorates or disappears in the different parts of *Lagerstroemia flos- reginae* kept in the laboratory under ordinary conditions. The rate of deterioration for every 20 gms of the dried parts of *Lagerstroemia flos- reginae* per weeks in approximately 0.15 units for fruit, 0.58 unit for flower; 0.6 unit for young leaves, and 0.9 unit for mature leaves. Since there were much studies carried out using only extracts of the seeds in experimental models, the present study was planned to evaluate the anti – diabetic potentiality of the leaves of *Lagerstromia speciosa.* Studies were also being planned to note the effect of seeds on other biochemical parameters and toxicity in experimental animals.

2. Materials and Methods

2.1. Plant Material

The leaves were collected, cleaned, shade dried and powdered. The alcoholic extract of *Lagerstroemia flos - reginae* leaves were prepared and administered to the rat orally by using a glass syringe.

2.2. Preparation of Alcoholic Extract

Air dried powdered plant leaves were taken in a round bottom flask and ethanol was added to it. Then it was refluxed over a heating mantle. The extract was filtered, distilled repeatedly to remove alcohol and finally the weight of the substance was determined. 5 gm was taken and suspended in 1% gum acacia and made up to 50 ml. This was then given to rats orally in doses of 1000 mg/kg body weight and 2000 mg/kg body weight.

2.3. Preparation of Standard Drug

In order to compare the results with the standard drugs in market, we have chosen Daonil – 5 mg / tablet and prepared the standard concentration of about 5mg/ kg body weight and given to the alloxan rats by diluting the concentration needed to give in relation to their body weights using distilled water orally.

2.4. Preparation of Diabetic Rats

Rats were made diabetic by injecting alloxan monohydrate intraperitoneally at a dose of 150 mg/kg body weight (in citrate buffer pH 4.5). After 72 hours blood was collected by retino orbital sinus puncture under mild ether anaesthesia of all surviving rats. Blood collected is Centrifuged at 3000 rpm within 1 hour and blood glucose levels were determined by using Autoanalyser Micro lab 2000. Serum glucose estimated by enzymatic method using ETPL Kits.

2.5. Estimation of Glucose[9]

The estimation was carried out in serum by using diagnostic kit supplied by ETPL. 10 µl (0.01 ml) of glucose solution was taken in the 'Standard' tube and 10 µl (0.01 ml) of serum was taken in the 'test' tube. 1000 µl (1.0 ml) of working enzyme reagent was added in 'blank', 'standard' and 'test' tubes. All the tubes were mixed well and incubated at 37°C for 10 minutes . Read the absorbance of 'standard' and 'test' against the blank at 505 nm.

2.6. Estimation of Triglyceride (GPO – POD-ESPAS method)[10]

The estimation was carried out in serum by using diagnostic kit supplied by ETPL Reagents must be brought to room temperature 10 µl (0.01 ml) of glycerol standard and 10 µl (0.01 ml) of serum was taken in the 'Test'. 1000 µl (1 ml) of working enzyme reagent was taken in blank, standard and test. Mixed well and incubated at 37°C for 10 minutes. Read the absorbance of standard and test against blank at 546 nm.

2.7. Estimation of Cholesterol[11]

10 µl (0.0 ml) of cholesterol standard was taken in the

standard tube and 10 µl (0.0 ml) of serum was taken in the 'test' tube. 1000 µl (1 ml) of working reagent was added in the 'blank', 'standard' and 'test' tubes. All the tubes were mixed well and incubated at 37°C for 10 minutes. Read the absorbance of standard and sample against blank, at 505 nm.

2.8. Estimation Of HDL Cholesterol[12]

Reagents must be brought at room temperature before use. To a test tube add 0.2 ml (200 µl) of sample and 0.2 ml (200 µl) of precipitating reagent. Mix well, and allow to stand for 10 minutes at room temperature. Centrifuge at 3000 rpm for 10 minutes to get clear supernatant. Then, the level of cholesterol is estimated as per the procedure of total cholesterol, reported earlier.

2.9. Estimation Of Uric Acid[13]

The working solutions must be brought at $^{+}$15 to $^{+}$25°C before use. Take 3 clean test tubes and mark it as 'Blank', 'standard' and 'Test'. To the tube marked 'Blank', add 25 µl of sample and 1000 µl of working solution. To the 'standard' add 25 µl of standard and 1000 µl of working solution. To 'Test' add 25 µl of sample and 1000 µl of working solution. Mix and incubate for 10 minutes at 37°C. Read the absorbance of the sample and of the standard against the blank at 546 nm.

2.10. Estimation of Bilirubin (Jendrassik – Grof Method)

Bilirubin reacts with diazotised sulphanilic acid (DSA) to form a red azodye. The absorbance of this dye at 546 nm is proportional to the bilirubin concentration in the sample. Water soluble bilirubin glucuronides react directly with DSA where as the albumin – conjugated indirect bilirubin will only react with DSA in the presence of an accelerator.

2.11. Tissue Estimation

A 5% homogenate of liver tissue was prepared in 0.1 m This HCL buffer (PH – 7.5) centrifuge the homogenate and the filterate is taken for the estimation of lipid peroxide, glutathione, and protein.

2.13. Estimation of Lipid Peroxide

TBARS (Thio-Barbituric Acid Reactive Substrate) was estimated by the thiobarbituric acid assay method of Nichans and Samuelson (1968). 1ml of homogenate was combined with 2 ml of TCA-TBA- HCL reagent and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the mixture was shaken with 4 ml of n-butanol to extract the coloured complex into the organic phase. The absorbance of the butanol layer was read at 535 nm against a blank that does not contain the sample. The concentration of malondialdehyde (TBARS) can be estimated using an extinction of $1.56 \times 10^5 m^{-1}\ cm^{-1}$.

2.14. Estimation of Glutathione

Placed 0.2 ml sample, 3 ml of precipitating solution and mixed. Allowed to stand for 5 minutes at room temperature and filtered through course grade filter paper. 2.0 ml supernatant was taken at test solution. 1.2 ml precipitating reagent was taken as blank, made up to 2 ml with distilled water and to this add 8ml phosphate buffer and 1ml DTNB solution. Mix well and read absorbance at 412 nm within 4 minutes. Reduced glutathione was taken as the standard and treated as test.

2.15. Estimation of Proteins in Liver Homogenised

Protein concentration was estimated by the method of Lowry *et al*.[14] using bovine serum albumin as a standard protein. Graded volumes of protein solution are pipetted out into a series of test tubes and made up to 1 ml using distilled water 0.25 ml of the test solutions was pipetted out in to a series of test tubes and made upto 1ml using distilled water. 5 ml of alkaline copper reagent was pipetted out in to all the tubes. It is mixed well and allowed to stand at room temperature for 10 minutes 0.5 ml of Folin's reagent was added to all the tubes. Mixed well and incubated at room temperature for 30 minutes optical density was read at 620 nm.

3. Results

The main objective of the study was to assess the antidiabetic effect of *Lagerstroemia flos-reginae* in terms of control of glucose level and other biochemical parameters such as lipid profile, uric acid, bilirubin in serum and lipid peroxides and glutathione in Liver following administration for 10 days, and the effect compared to a standard drug Daonil. In the tables presented group II is compared to group I and groups III, IV and V is compared with group II.

A significant increase in the level of glucose ($P<0.01$)

Table 1. Effect on glucose and lipid profile in serum
Effect of *Lagerstroemia flos-reginae* on the levels of serum glucose of Lipid profile in Diabetic rats. Values are mean 1 Standard Deviation of six rats in each group and expressed in mg%

Group	Sugar	TG	TC	HDL	LDL	LDL/HDL (Ratio)
Normal	74.3±10.3	63.7±11.8	88±6.7	23.8±4.5	51.4±3.4	2.1
Diabetic	*** 466±92.8	*** 182±18.4	*** 150±19.9	28.6±2.9	86±3.7	3.07
Diabetic + drug-1	*** 128±18.4	** 83.5±10.3	*** 91±9.9	25±3.1	49.7±2.8	1.95
Diabetic + drug2	*** 82.5±21	** 100±11.5	** 99±6.4	26±2.4	53±2.9	2.03
Diabetic + Daonil	*** 77±19	** 94±9.8	*** 86±8.1	22.8±1.4	45±2.9	2.04

P < 0.01*** - Most Significant (MS)
P < 0.02** - Significant (S)
P < 0.05* - Least Significant (LS)
P - Value denotes significance

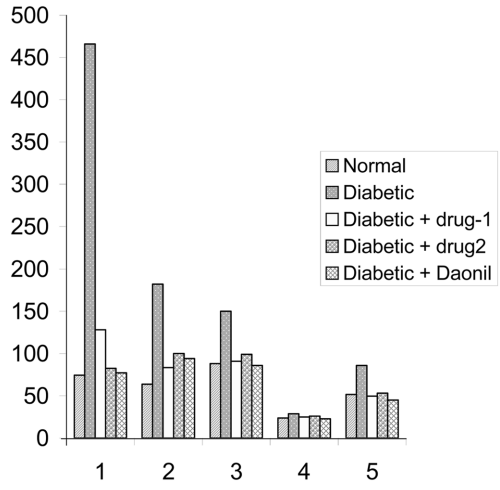

Fig. 1. Effect of *Lagerstroemia flos-reginae* on the levels of serum glucose of Lipid profile in Diabetic rats. Values are mean 1 Standard Deviation of six rats in each group and expressed in mg%

was observed in alloxan diabetic rats compared to normal glycemic rats. The diabetogenic effects of alloxan are variable during the first 48 hrs after administration of alloxan changes in glucose is triphasic consisting of initial hyperglycemia followed by permanent glycemia. Catherine, *et al*.[15] reported that induction of diabetes in rats by a single intraperitoneal injection of alloxan reduces the mortality of the rats. Insulin deficiency leads to various metabolic aberrations in the animals namely increased blood glucose, cholesterol and triglycerides. Thus a significant increase in the glucose level was observed in the serum of alloxan diabetic rats (group II) compared to normal glycemic rats (group I) ($P < 0.01$). Administration of *Lagerstroemia flos-reginae* leaf extracts caused a significant reduction in the blood sugar level in diabetic rats. The hypoglycemic effect of *Lagerstroemia flos-reginae* – both the doses (500 mg and 1000 mg per kg body wt). Groups III and IV were significant ($P<0.01$). Similar activity was observed in the daonil treated (group V) rats. The possible mechanism of reduction in the *Lagerstroemia flos-reginae* treated group of rats may be due to the capacity of the test – drug extracts to induce pancreatic secretion of insulin from the β-cells or due to enhanced transport of blood glucose to the peripheral tissues or due to reduced glucose absorption from the gastro – intestinal tract. Many plants reported to be useful for the treatment of diabetes mellitus in ayurvedic medicine have been tested by Chopra, *et al*., for their hypoglycemic activity in experimental animals.

The blood sugar reduction caused by the decoction of *Lagerstroemia flos-reginae* leaves were relatively greater when the initial blood sugar was higher than when the initial amount was low as said by Garcia[16] from Phillipines.

A significant increase in the level of serum triglycerides and total cholesterol was found in the diabetic rats compared to normal control and *Lagerstroemia*

Table 2. Effect of *Lagerstroemia flos-reginae* on the levels of lipid peroxides and glutathione in liver and bilirubin and uric acid in the serum of experimental rats

Group	Lipid Peroxides n moles/mg/ptn	Glutothione mg/g ptn	Uric acid mg%	Bilirubin mg %
Normal	0.8±0.19	3.6±0.53	4.4±0.5	0.68±0.1
Diabetic	*** 2.299±0.22	** 1.7±0.45	4.5±0.4	0.78±0.1
Diabetic + drug-1	** 1.41±0.21	2.6±0.54	4.6±0.42	0.76±0.09
Diabetic + drug2	*** 1.25±0.18	** 3.2±0.33	3.35±0.34	0.78±0.11
Diabetic + Daonil	*** 1.1±0.17	*** 3.4±0.28	3.53±0.11	0.76±0.11

P < 0.01*** - Most Significant (MS)
P < 0.02** - Significant (S)
P < 0.05* - Least Significant (LS)
P - Value denotes significance

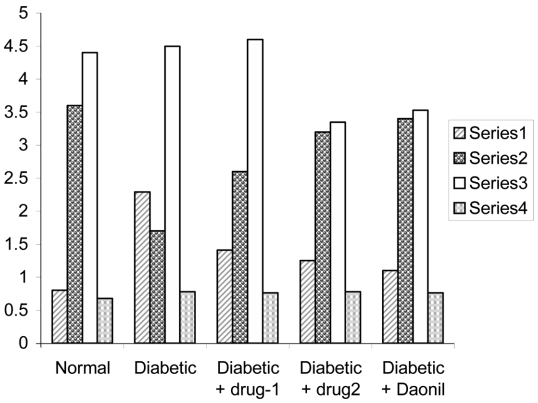

Fig. 2. Effect of *Lagerstroemia flos-reginae* on Lipid peroxides and glutothione in liver and Bilirubin and Uric acid in serum of alloxan diabetic rats. Values are mean ± Standard Deviation of six rats in each group.

flos-reginae treated diabetic rats. The increase in the cholesterol level in diabetic rats may be due to increased cholesterolgenesis and hyper triglyceridemia observed may be due to lack of insulin to activate the enzyme lipoprotein lipase. Mitre, *et al*., have reported a lowering of Triglycerrides and improvement in the HDL cholesterol in streptzotocin diabetic rats treat with a polyherbal formulation D-400 containig *Cucurma longa*. The levels of cholesterol and triglycerrides were significantly reduced in *Lagerstroemia flos-reginae* treated diabetic rats and the results are comparable to the action of daonil in the alloxan diabetic rats which confirms the insulin like activity of *Lagerstroemia flos-reginae*. Bhatt, *et al*.[17] had observed an inverse relationship between the HDL cholesterol and LDL cholesterol. The results show an alteration in the metabolism of lipids as observed by Mitre, *et al*.[18]. The ratio of LDL cholesterol to HDL cholesterol was increased in the alloxan diabetes rats showing an increase in LDL – cholesterol and a decrease in HDL cholesterol. Like daonil, *Lagerstroemia flos-reginae* could effectively reverse the ratio to that seen in normal rats thereby confirming the anti-hyperlipidemic activity of the active principles of the *Lagerstroemia flos-reginae* leaf extracts and offering protection.

The level of lipid peroxides increased and that of glutathione decreased in diabetic rats when compared to control group of rats. *Lagerstroemia flos-reginae* in both the doses administered to diabetic rats caused a reversal of both lipid peroxides and glutathione. The decrease in lipid peroxides and increase in glutathione was also seen in the daonil treated group. The impairment of the anti- oxidant system can be correlated to the increase in lipid peroxides in the diabetic rats which indicates the production of free radicals causing toxic insult of the liver tissue. This results in the damage of liver tissue. This results in a marked decline in the 'non-enzymatic anti- oxidants resulting in an increase in lipid peroxides an decrease in glutathione in the diabetic rats. The decrease in lipid peroxides in the drug treated group shown the ability of the active principles of *Lagerstro-*

emia flos-reginae to quench and scavenge the toxic products of lipid peroxidation have reported that glutathione and vitamin E were reduced in untreated diabetic rats compared to a two fold increase in glutathione in animals treated with fenugreek. Similar effect was seen in the glutathione content of alloxan diabetic and *Lagerstroemia flos-reginae* treated group. In the present study *Lagerstroemia flos-reginae* has effectively maintained the glutathione content and terminated lipid peroxidation thereby preventing tissue damage and raising the anti oxidant status. The levels of uric acid and bilirubin showed no change in both diabetic and drug treated group may be due to short period of study and the change may be latent.

The study shows that *Lagerstroemia flos-reginae* is able to correct the metabolic aberrations of diabetic rats to a certain extent where only insulin could control the metabolic path ways. The mechanism of action may be due to its insulin like activity by the biochemical parameters to bringing the control level showing the presence of anti-diabetic principle in the leaf extract of *Lagerstroemia flos-reginae.*

4. Conclusion

Alloxan treated diabetic rat's shows alterations in all the Biochemical parameters studied. The elevated blood sugar was brought to normal level by the administration of *Lagerstroemia flos- reginae* and the normalization indicates the efficiency of the drug in the treatment of diabetes. Alloxan diabetes causes liver damage due to free radical formation during its metabolism by hepatic microsomes, which in liver causes peroxidation of cellular membranes leading to necrosis of hepatocytes.

Lagerstroemia flos-reginae treated diabetic rats could reverse the changes in the biochemical parameters, the active principles present in the leaves extracts of *Lagerstroemia flos-reginae* were able to correct the metabolic aberrations to some extent where only insulin controls the metabolic pathways. Thus it was possible to evaluate the anti-diabetic, anti hyperlipidemic and anti-oxidant potential of *Lagerstroemia flos-reginae* on alloxan diabetic rats. This study hence peroxides forms a basis for further detailed investigation on the therapeutic efficiency of the *Lagerstroemia flos-reginae* for confirming the anti – diabetic potential claimed in ethanopharmocology.

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