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Evaluation of antioxidant and antidiabetic activity of hydroalcoholic leaves extract of *lagerstroemia floribunda*

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ABSTRACT

Type 1 and type 2 diabetes mellitus is a serious and lifelong condition commonly characterised by abnormally elevated blood glucose levels due to a failure in insulin production or a decrease in insulin sensitivity and function. Over the years, prevalence of diabetes has increased globally and it is classified as one of the leading cause of high mortality and morbidity rate. Furthermore, diabetes confers a huge economic burden due to its management costs as well as its complications are skyrocketing. The conventional medications in diabetes treatment focusing on insulin secretion and insulin sensitisation cause unwanted side effects to patients and lead to incompliance as well as treatment failure. Besides insulin and oral hypoglycaemic agents, other treatments such as gene therapy and induced β-cells regeneration have not been widely introduced to manage diabetes. Secondary Plant constituents contain alkaloids, flavonoids, phenol, saponin, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. In Indian Ayurvedic system, *Lagerstroemia floribunda* are well-known plants used for major and minor ailments. The aim of the present study is to examine leaf of *Lagerstroemia floribunda* for phytochemical profile, antioxidant potential and diabetic activity. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by FolinsCiocalteau reagent method and aluminium chloride method respectively. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Lagerstroemia floribunda*, Diabetes mellitus**,** β-cells, Antioxidant, Antidiabetic.

1. INTRODUCTION

1.1 Diabetes mellitus

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both.

Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both. Classification of diabetes mellitus is based on its aetiology and clinical presentation. As such, there are four types or classes of diabetes mellitus viz; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types. Type 1 diabetes is said to account for only a minority of the total burden of diabetes in a population although it is the major type of the diabetes in younger age groups at majority of well-to-do countries.

The incidence of type 1 diabetes is increasing in both rich and poor countries. Furthermore, a shift towards type 1 diabetes occurring in children at earlier ages is imminent. 85 to 95% of all diabetes in high-income countries is of type 2 accounting for an even higher dominance in developing countries. It is intimately associated with improper utilization of insulin by target cells and tissues. It is currently a common and serious health concern globally. According to WHO this problem has been aggravated by rapid cultural and social dynamics, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns. Diabetes mellitus and lesser forms of glucose intolerance, particularly impaired glucose tolerance, can now be found in almost every population in the world and epidemiological evidence suggests that, without effective prevention and control programmes, diabetes will likely continue to increase globally.

In 2010, about 285 million people in the age group 20-79 were envisaged to have diabetes worldwide, about 70% of whom live in developing nations. This estimate is expected to increase to about 438 million, by 2030. Further, by 2030, the number of people with IGT is projected to increase to 472 million, or 8.4% of the adult population. The debilitating effects of diabetes mellitus include various organ failures, progressive metabolic complications such as retinopathy, nephropathy, and/or neuropathy. Diabetics are accompanied by risk of cardiovascular, peripheral vascular and cerebrovascular diseases. Several pathogenetic processes are involved in the development of diabetes, including destruction of pancreatic β-cells that lead to lowered sensitivity of insulin action.

1.2 Classification

A. Type 1 diabetes mellitus

The American Diabetes Association provides clear definitions of the various types of diabetes and classification, diagnosis, and clinical care of diabetes. Type 1 DM, which results from destruction of beta cells in the pancreas, accounts for approximately 10 percent of all patients with DM in the United States. It leads to absolute insulin deficiency. There are two forms of type 1 DM. One is an immune-mediated disease with autoimmune markers such as islet cell antibodies (ICAs), insulin autoantibodies (IAAs), and autoantibodies to glutamic acid decarboxylase (GAD). As many as 85 − 90 percent of patients with fasting

hyperglycemia are positive for one or more of these markers. Strong human leukocyte antigen (HLA) associations also exist. A second form of type 1 DM, now called idiopathic diabetes, has no known cause.

Only a minority of patients fall into this group, which occurs mainly in individuals of African and Asian origin. Idiopathic diabetes is strongly heritable, but it lacks autoimmune markers and is not HLA associated. Although it can occur at any age, type 1 DM is more common in persons less than 30 years of age. The rate of pancreatic destruction is variable and is generally more rapid in infants and children and slower in adults.

B. Type 2 diabetes mellius

Type 2 is the most common form of DM worldwide, and its prevalence is increasing. Its underlying defects can vary from predominant insulin resistance with relative insulin deficiency to a predominant insulin secretory defect with insulin resistance. A great deal of heterogeneity exists, and most patients with type 2 DM do not initially require insulin therapy. Accounting for approximately 90 percent of all cases of DM in the United States, type 2 DM occurs more frequently in adults than in children, and the incidence increases with age, especially after age 40. However, the prevalence of type 2 DM in children is increasing, especially in the high-risk ethnic groups, such as Native Americans, Hispanic Americans, African Americans, and Asian Americans. Most of these children are between 10 and 19 years old, have had symptoms longer, have infrequent or mild diabetic ketoacidosis, are obese, and have a strong family history of diabetes. A characteristic finding is darkening of the skin (acanthosis nigricans) and there is an increased incidence of insulin resistance.

1.3 Signs and symptoms

1.3.1 Symptoms of Type 1 Diabetes

1. Frequent urination
2. Unusual thirst
3. Extreme hunger
4. Unusual weight loss
5. Extreme fatigue and Irritability

There is a reason why diabetes is termed the silent killer. It is important to bear in mind that these symptoms may be mistaken for an ailment in themselves or for some other disease. The best method to diagnose this condition is to have a blood test taken. And if you have already noticed this symptom, you should see a doctor at the earliest.

1.3.2 Symptoms of Type 2 Diabetes

1. Excessive Urination and Thirst
2. Increased Hunger
3. Unexplained Weight Gain
4. Irritability and Fatigue
5. Blurred Vision
6. Warning Signs of Diabetes
   a) Decelerated Healing
   b) Skin and Yeast Infections plus Frequent Gum and Bladder Infections

1.3.3 Other Symptoms

1. Sexual Dysfunction in Men
2. Vaginal Infections in Women
3. Numbness/Tingling in hands and feet
4. Itchy or Flaky Skin.

2. MATERIALS AND METHOD

2.1 Collection of plant material

Leaves of *Lagerstroemia floribunda* was collected from riral area of Dist. Raisen.

2.2 Extraction procedure by maceration process

100 gm of dried powdered Leaves of *Lagerstroemia floribunda* has been extracted with hydroalcoholic solvent (ethanol:water:70:30) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40^0C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

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2.4 Estimation of total Phenolic and flavonoid Content

2.4.1 Total Phenolic content estimation

Procedure: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared

in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

2.4.2 Total flavonoids content estimation

Procedure: Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.5 Antioxidant activity of extracts by Nitric oxide scavenging activity

Sodium nitroprusside formed nitrogen oxide, and weighed the Griess reagent. Sodium nitroprusside naturally generates nitric oxide in aqueous solution at physiological pH, reacting with oxygen to create nitric ions which can be measured with the Griess reagent. Nitric oxide scavengers interact with oxygen, resulting in reduced nitric oxide output. In the phosphate buffer saline (PBS), sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations and incubated for 150 min at 25 ° C. Griess reagents (1% sulphanilamide, 2% H_3PO_4, and 0.1% napthylethylenee di amine dihydrochloride) were added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethylenee diamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. Both triplicate tests were performed, and mean values were plotted on the table. The proportion of inhibitions was measured using the following formula:

$$\textbf{Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{x}100$$

Where control absorbance is the absorption (without extract) of the control and where test absorbance is the absorption in the presence of the extract / standard.

2.6 *In -Vivo* Anti diabetic activity

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All

the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments.

Induction of Experimental Diabetes in Rats

After fasting, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of 'Alloxan monohydrate' in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. These animals were tested for diabetes after 15 days and animals with blood glucose (fasting) were selected for experimentation.

2.7 Experimental Protocol

Animals were divided into five groups of 6 rats each.

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

Group II: Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

Group III: Rats (diabetic) were administered of hydroalcoholic leaves extract of *Lagerstroemia floribunda* (100 mg/kg p.o.) for 15 days

Group IV: Rats (diabetic) were administered of hydroalcoholic leaves extract of *Lagerstroemia floribunda* (200 mg/kg p.o.) for 15 days

Group V: Rats (diabetic) were administered Glibenclamide (600µg/kg p.o.) for 15 days.

3. RESULTS AND DISCUSSION

3.1 Determination of Percentage Yield

Yield of Extraction: The crude extract so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvent completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using Pet. ether, hydroalcoholic as solvents are depicted in the table 3.1.

Table 3.1: % Yield of Leaves of *Lagerstroemia floribunda*

S. No.	Solvents	% Yield
1.	Hydroalcoholic	8.92

3.2 Result of Phytochemical screening of extracts *of* Leaves of *Lagerstroemia floribunda*

Table No.3.2 Phytochemical screening of extracts *of* Leaves of *Lagerstroemia floribunda*

S. No.	Constituents	Methanol extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	 -Ve +Ve
2.	Glycosides A) Legal's Test:	 +Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	 +Ve +Ve
4.	Saponins A) Froth Test:	 +Ve
5.	Phenolics A) Ferric Chloride Test:	 +Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	 -Ve
7.	Carbohydrate A) Fehling's Test:	 +Ve
8.	Diterpenes A) Copper acetate Test:	 -Ve
9.	Tannin A) Gelatin test:	 +Ve

3.3 Results of estimation of total phenolic contents Total flavonoid content of Leaves of *Lagerstroemia floribunda*

Table no. 3.3 Total phenolic contents Total flavonoid content of Leaves of *Lagerstroemia floribunda*

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic *extract*	4.95	3.81

3.4 Results of antioxidant activity using NO method

The NO scavenging activity of hydroalcoholic extract of *Lagerstroemia floribunda* was 69.92 µg/ml, IC50 value respectively. This activity was lower than ascorbic acid, reference standard ascorbic acid showed IC_{50} 29.017µg/ml.

3.5 Results of *in vivo* anti diabetic activity

Table 3.4: Effect of hydroalcoholic Leaves extract of *Lagerstroemia floribunda* treatment on blood glucose (mg/dl) in normal and diabetic rats

Group	Treatment	Blood glucose (mg/dl)		
		Days 0	Days 8	Days 15
I	Normal	91.60± 5.50	96.20 ± 5.50	100.73 ± 5.50
II	Diabetic Control	277.50 ± 10.35	289.10± 9.35$^{\#}$	294.50± 8.35$^{\#}$
III	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda* (100 mg/kg)	256.50 ± 4.50	161.50± 4.10***	127.50 ± 3.50***
IV	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda*(200 mg/kg)	251.52 ± 3.00	151.70 ± 4.00***	118.90 ± 4.45***
V	Diabetic + Glibenclamide (600µg/kg)	255.00 ± 4.90	134.10± 4.90***	112.35 ± 4.90***

Figure 3.1: Effect of hydroalcoholic extract of *Lagerstroemia floribunda* treatment on blood glucose (mg/dl) in normal and diabetic rats

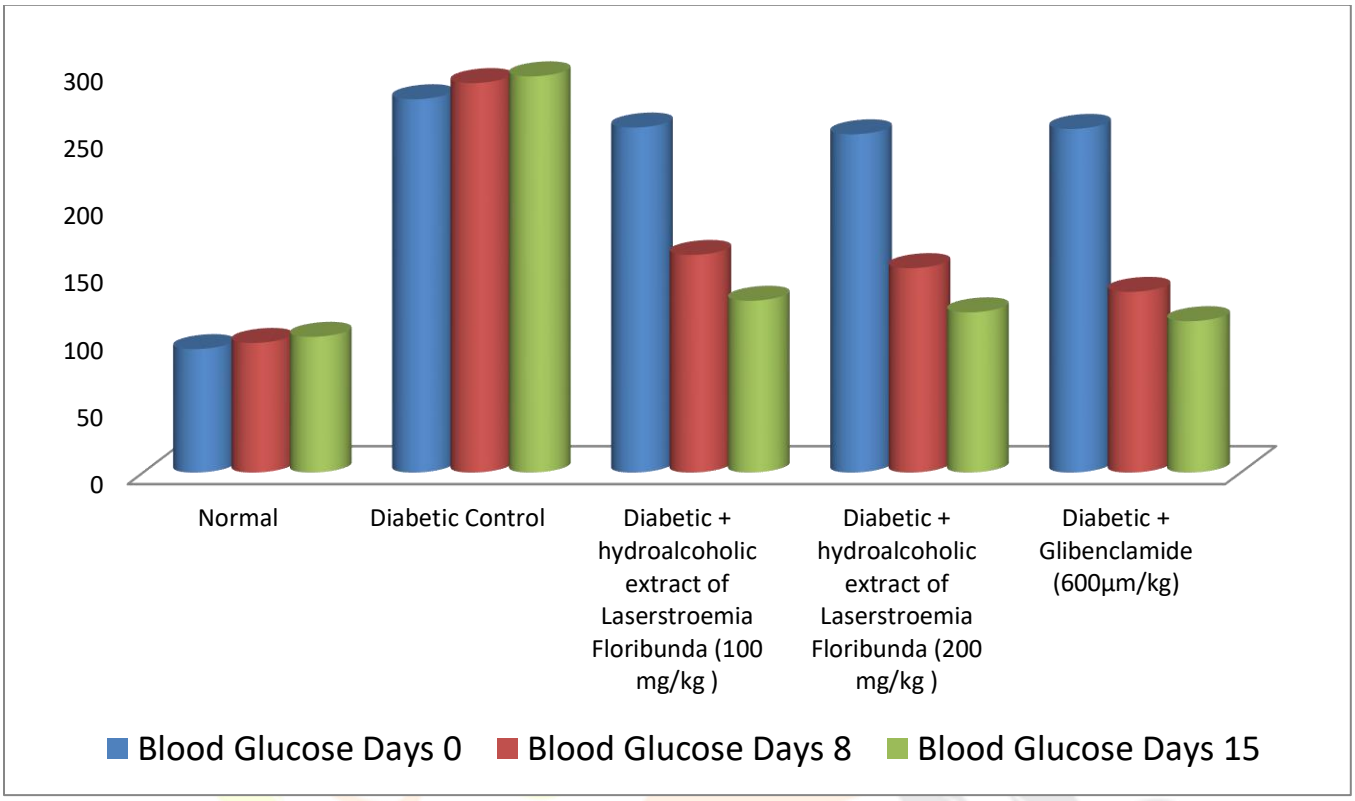


Table 3.5: Effect of hydroalcoholic extract of *Lagerstroemia floribunda* treatment on biochemical parameters in normal and diabetic rats

Group	Treatment	TC (mg/dL)	TG (mg/dL)	Total protein(g/dl)
I	Normal	92.50 ± 3.00	86.90 ± 3.00	9.00 ± 1.50
II	Diabetic Control	181.0 ± 5.00	122.8 ± 6.00	4.80 ± 1.50
III	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda* (100 mg/kg)	110.8 ± 5.15**	88.10 ± 6.00*	8.15 ± 2.50**
IV	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda* (200 mg/kg)	106.5 ± 5.10**	85.17 ± 6.00*	8.53 ± 2.50**
V	Diabetic + Glibenclamide (600µg/kg)	100.3 ± 5.10**	81.90 ± 6.00*	8.38± 2.50**

Figure 3.2: Effect of hydroalcoholic extract of *Lagerstroemia floribunda* treatment on total cholesterol in normal and diabetic rats

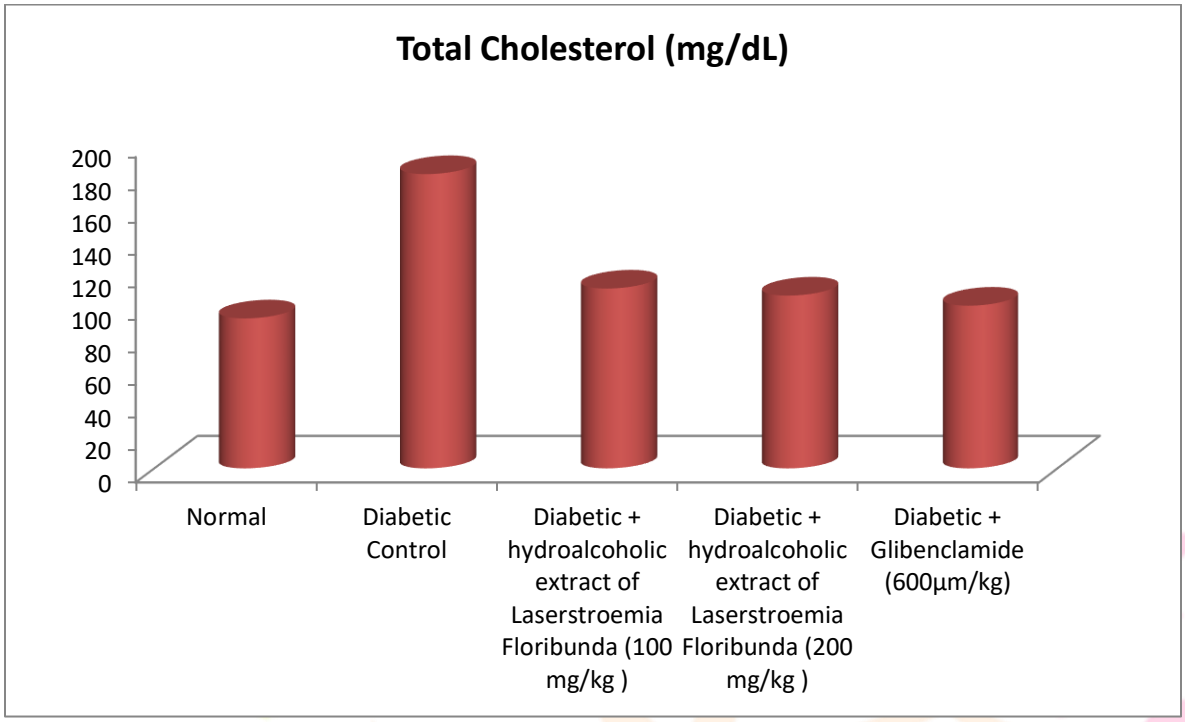


Figure 3.3: Effect of hydroalcoholic extract of *Lagerstroemia floribunda* treatment on triglyceride in normal and diabetic rats

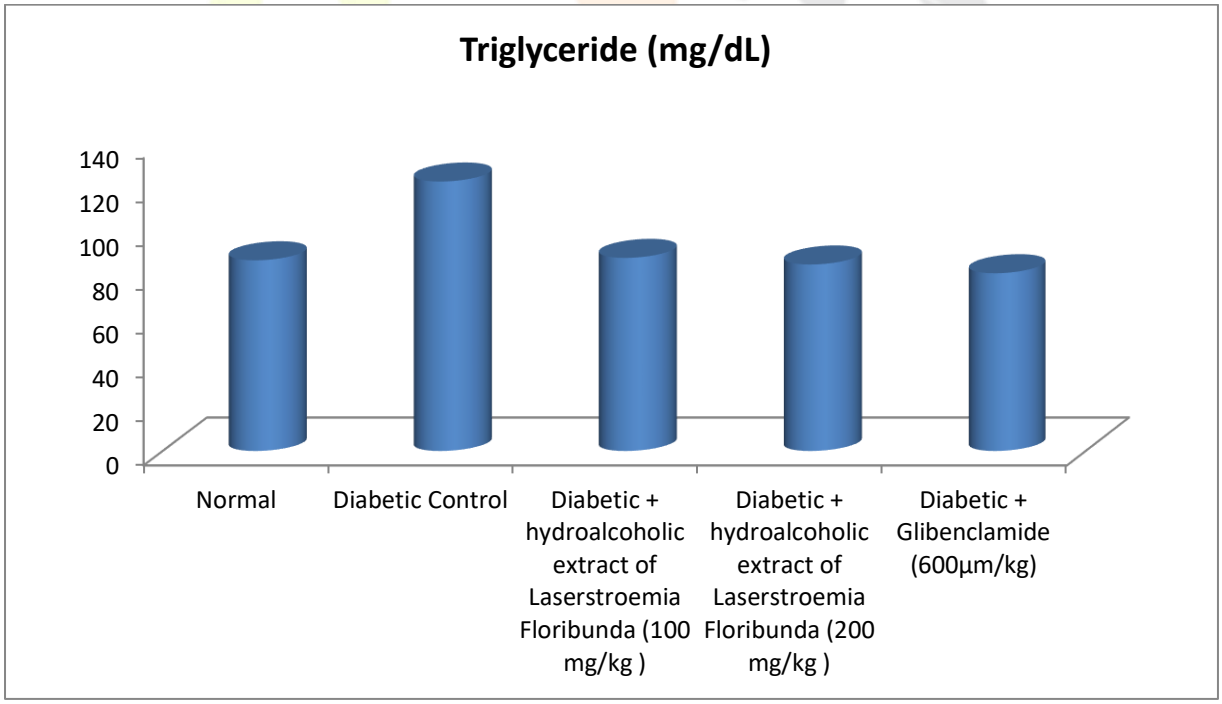


Figure 3.4: Effect of hydroalcoholic extract of *Lagerstroemia floribunda* treatment on total protein in normal and diabetic rats

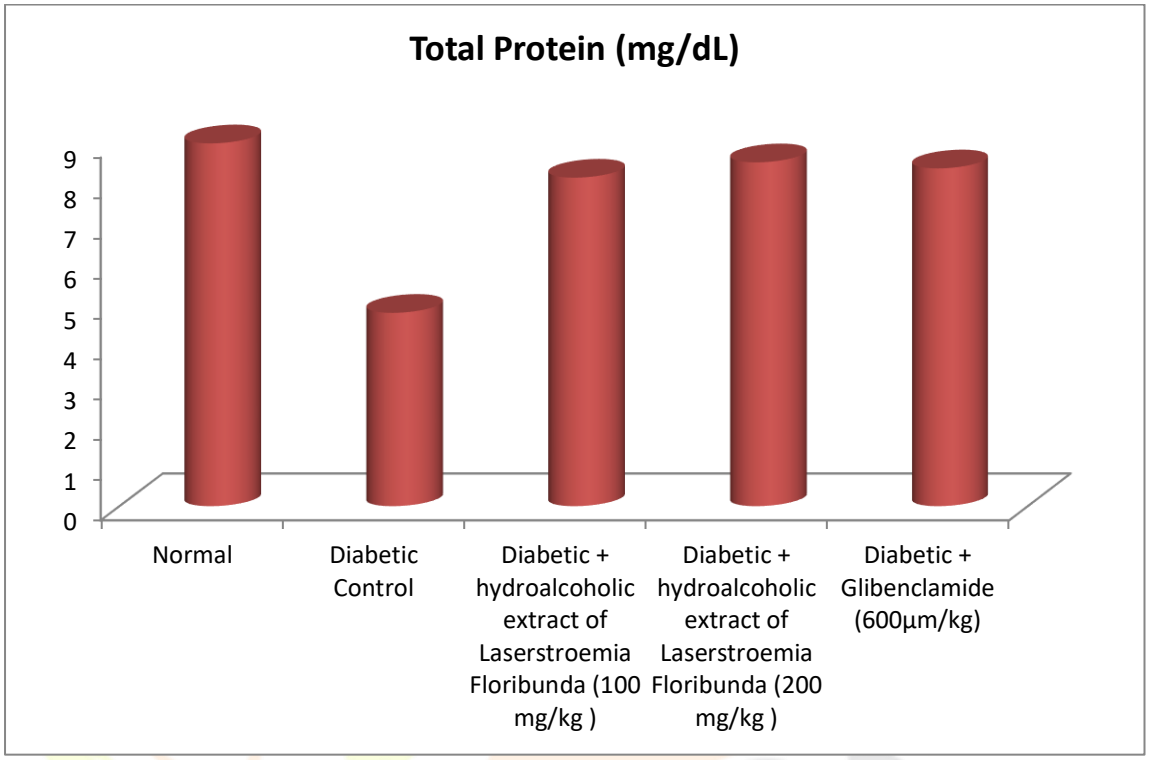
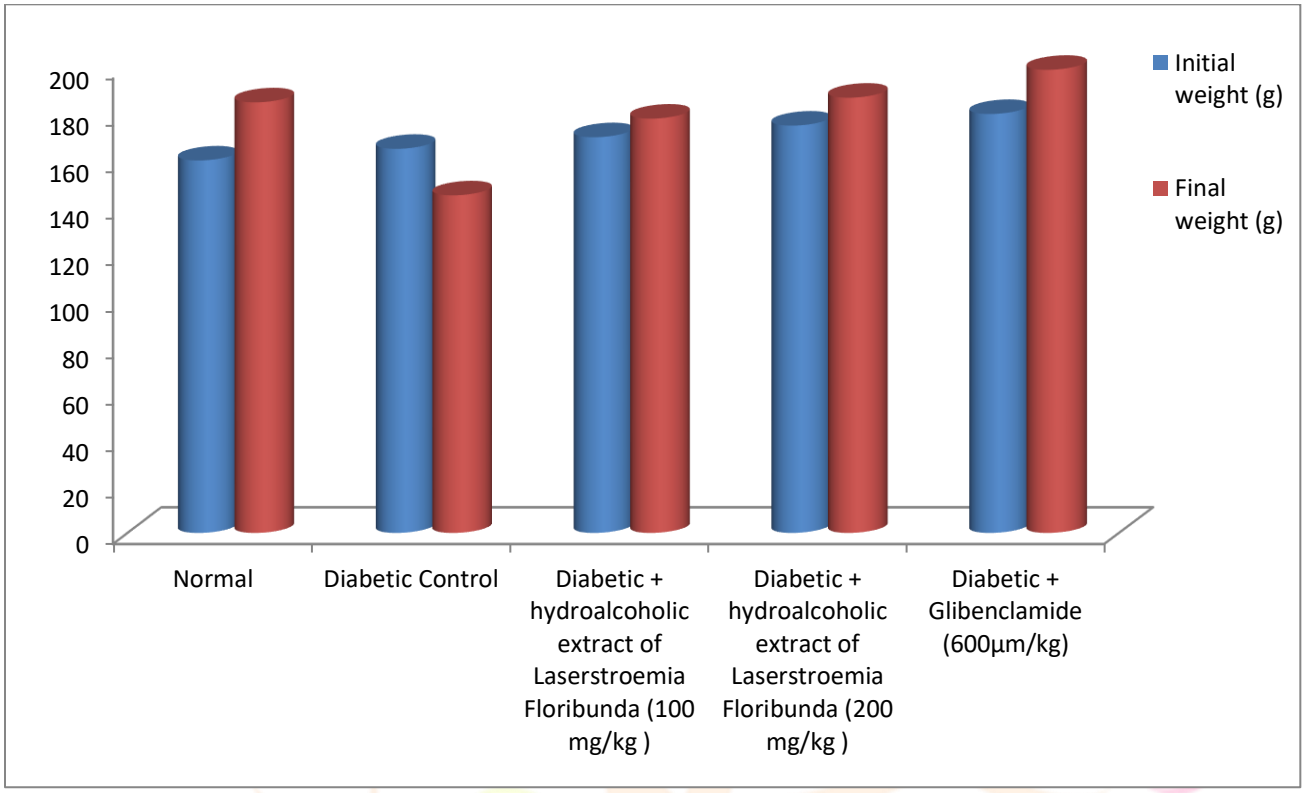


Table 3.6: Effects of hydroalcoholic extract of *Lagerstroemia floribunda* on body weight

Group	Treatment	Initial weight (g)	Final weight(g)
I	Normal	160.00 ± 8.00	185.10 ± 9.00
II	Diabetic Control	165.00 ± 8.00	145.00 ±9.00
III	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda* (100 mg/kg)	170.00 ± 8.00	178.50 ± 8.00
IV	Diabetic + hydroalcoholic extract of *Lagerstroemia floribunda* (200 mg/kg)	175.00 ± 8.00	187.25 ± 9.00
V	Diabetic + Glibenclamide (600µg/kg)	180.00 ± 8.00	199.75 ± 9.00

Figure 3.5: Effects of hydroalcoholic extract of *Lagerstroemia floribunda* on body weight



CONCLUSION

The current study aimed to establish the mechanisms of antidiabetic activity of hydroalcoholic extract of *lagerstroemia floribunda* leaves in 'Alloxan monohydrate'-induced type 2 diabetes in rats. Phytochemical screening, and acute toxicity study of of hydroalcoholic extract of *lagerstroemia floribunda* leaves were carried out. Concentrations of hydroalcoholic extract of *lagerstroemia floribunda* leaves (100 and 200 mg/kg) were administered orally to diabetic rats.. The antidiabetic effect of of hydroalcoholic extract of *lagerstroemia floribunda* leaves was examine by measuring blood glucose levels in rats. The antioxidant effect of hydroalcoholic extract of *lagerstroemia floribunda* leaves was determined by Nitric oxide scavenging activity method.

In the results of our study indicates that of hydroalcoholic extract of *lagerstroemia floribunda* leaves has good antidiabetic activity. Hydroalcoholic extract of *lagerstroemia floribunda* leaves exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight. They can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile. The renewal of cells in diabetes has been studied in several animal models. The total cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet ß cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug. Hydroalcoholic extract of *lagerstroemia floribunda* leaves has been shown to act by cell regeneration. In our studies, the damage of pancreas in

alloxan-treated diabetic control rats and regeneration of cells by glibenclamide was observed. It is found that of hydroalcoholic extract of *lagerstroemia floribunda* leaves at high dose (200 mg/kg) is more effective than plant extract at low dose (100 mg/kg) after 15 days of treatment. Hence the above discussion revels that of hydroalcoholic extract of *lagerstroemia floribunda* leaves at high dose (200 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (600 µg/kg). This could be due to the possibility that some -cells are still surviving to act upon by hydroalcoholic extract of *lagerstroemia floribunda* leaves to exert its insulin releasing effect.

From the above discussion it conclude that Hydroalcoholic extract of *lagerstroemia floribunda* leaves at high dose (200 mg/kg) exhibited significant antihyperglycemic activity than whole plant extract at low dose (100 mg/kg) in alloxan-induced diabetic rats.

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