ORIGINAL ARTICLE

Antidiabetic evaluation of methanolic extract of *Lagerstroemia parviflora* Roxb. in streptozotocin-induced diabetic Wistar albino rat model

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Abstract

Objective: The objective of research paper was to evaluate the antidiabetic activity of methanolic extract of *Lagerstroemia parviflora* Roxb. (MELPR) in streptozotocin (STZ)-induced diabetic rats. **Materials and Methods:** The MELPR of different concentrations (200 and 300 mg/kg b.w.) was administered orally and evaluated for the estimation of biochemical parameters (serum glutamate pyruvate transaminase [SGPT], alkaline phosphatase [ALP]), skin and behavioral activity, oral glucose tolerance test and very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), VLDL cholesterol and LDL cholesterol (VLDL-C and LDL-C), and antidiabetic potential. **Results and Discussion:** The antidiabetic activities data indicate substantially altered physiological and biochemical aspects. In *Lagerstroemia parviflora* Roxb. (MELPR), the dose of 100 and 200 mg/kg shown the cholesterol level 138.0 mg/dl and 125.0 mg/dl as compared to standard drug glibenclamide which shown the cholesterol level 110.0 mg/dl at a dose of 500 mg/kg. Study result indicates that significant decrease in total cholesterol, triglyceride, and LDL cholesterol levels was observed after treating the STZ-induced diabetic rats with 200 and 300 mg/kg body weight leading to a significant increase in the cholesterol levels. **Conclusion:** In acute toxicity study, no toxic symptoms were observed for MELPR, up to dose 3000 mg/kg. The MELPR possesses hypoglycemic activity, which varies with concentration. In neutral and basic media, the hypoglycemic activity was considerably more than that in acidic media. This study demonstrates the antidiabetic potential of *Lagerstroemia microcarpa* Wight and offers scientific validation and basis to develop antidiabetic drug.

Key words: Antidiabetic, cholesterol, hypoglycemic, low-density lipoprotein, serum glutamate pyruvate transaminase, streptozotocin

INTRODUCTION

Today, we are witnessing a great deal of public interest in the use of herbal remedies. The WHO estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health-care needs and most of this therapy involves the use of plant extracts or their active components.[1]

Diabetes

The two main forms of diabetes are; type 1 diabetes mellitus (T1DM), which accounts for 10% of all diagnosed cases, and type 2 diabetes mellitus (T2DM), which is the more prevalent type, accounting for 90% of all diagnosed cases. Clinically, both types share some similarities, including presenting indications such as polydipsia or polyuria. However, due to their variances in etiology, they are considered to be at opposite ends of a spectrum in relation to insulin production and function.

In T1DM, patients are dependent on insulin due to the irreversible, cell-mediated destruction of the pancreatic beta

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cells, resulting in complete insulin deficiency. Therefore, the disturbance of insulin production in T1DM is the main feature of this type. T1DM usually has a juvenile onset and is associated with genetic susceptibility inherited through the major histocompatibility complex (HLA Class II region on chromosome 6p21) genes encoding DQ and DR.[2] The autoimmune response in susceptible type 1 individuals can be triggered by environmental factors[3] such as toxins or foods, resulting in the abnormal stimulation of B-cell- and T-cell-mediated immune response. This initiates the production of autoantibodies, including islet cell autoantibodies, insulin autoantibodies, autoantibodies to glutamic acid decarboxylase, and autoantibodies to tyrosine phosphatases (IA-2a and IA-2b)[4] against beta-cell antigens and triggers insulitis, with subsequent destruction of the pancreatic insulin producing beta-cells, creating absolute insulin deficiency.

However, unlike T1DM, patients with T2DM have preserved ability to produce insulin from their beta-cells, but due to insulin resistance, peripheral cells fail to respond to insulin. Therefore, it is the disturbance of insulin action from the diminished response of peripheral tissues to insulin in T2DM that is the main feature.[5] Albeit, T2DM patients also present with decreased beta-cell mass. This means that the remaining beta-cells have an increased workload, leading to further decline and failure of the beta-cells, resulting in subsequent loss of insulin secretion. There are various factors that contribute to the development of T2DM, and these include poor lifestyle choices and obesity, as well as genetic factors linked to beta-cell dysfunction.[6] The diminishing effects of insulin secretion and action in T2DM are related to increased levels of ectopic fat storage, increased secretion of adipokines, and elevated non-esterified fatty acids in circulation. T2DM was previously more commonly diagnosed in adulthood, with predisposition linked with ethnicity and family history.[7] However, age onset for this disorder has decreased, with incidence of T2DM increasing in children. This reflects the rise in childhood obesity,[8] along with other factors, including lack of physical activity and diets high in saturated fats.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Organoleptic characters, morphological characters, and microscopical examination were done to identifying crude drug. For identification of unknown drugs, herbariums and leading botanical gardens are of great help. The leaves of selected plant, namely, *L. parviflora* Roxb. were identified and collected from Bhimbetka Bhojpur, Bhopal (Madhya Pradesh [M.P.]), in the month of October 2019. The leaves were authenticated by expert Botanist of Council of scientific and Industrial Research (Delhi). The plant (leaves) was compared with voucher specimen (Ref. No. NISCAIR/RHMD/consult/2019 / 3558-59).

Animals

Wistar albino rats of both sexes (180–220 g) were used for antidiabetic and antiobesity study. The animals were obtained from the animal house of College of Veterinary Science and Animal Husbandry, Mhow, M.P. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the central animal house of College of pharmacy, SSSUTMS, Sehore. The animals were facilitated with standard environmental condition of photo period (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed and water given *ad libitum*. The use of these animals and the study protocols was approved by Institutional Animal Ethical Committee.

Selection of the Doses for Animal Study

The acute oral toxicity studies and selection of doses were carried out as per guidelines of Organization for Economic Cooperation and Development (OECD), draft guidelines 423 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy Wistar rats of either sex weighing between 180 and 220 g were used for acute toxicity study to determine LD50 of *L. parviflora* Roxb. The animals were randomly selected, marked to permit individual identification, and kept in their cages for 7 days before dosing to allow for acclimatization to the laboratory condition. In acute toxicity study, no toxic symptoms were observed for *L. parviflora* Roxb. up to dose 3000 mg/kg body weight. All animals behaved normally. No neurological or behavioral effect could be noted. No mortality was found up to 14 days study.

Antidiabetic Studies

Animal grouping for antidiabetic studies

Rats will be divided into different groups, each group consisting of six animals. After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in Groups II–VI by intraperitoneal injection of streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer at pH 4.5, at a dose of 55 mg/kg body weight. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia1. Diabetes status was confirmed by estimating blood glucose levels after 72 h of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for study.

Group	Treatment	No. of animals (*n*)
Group-I	Normal	6
Group-II	Diabetic control received only STZ (negative control)	6

Group-III	Diabetic rats received glibenclamide orally at dose of 500 mcg/kg b.wt for 14 days	6
Group-IV	Diabetic rats received methanolic extract of *Lagerstroemia parviflora* Roxb. (200 mg/kg/day p.o.)	6
Group-V	Diabetic rats received methanolic extract of *Lagerstroemia parviflora* Roxb. (300 mg/kg/day p.o.)	6
Total no. of animal used for the study		30

Biochemical analysis

Body weight of the experimental rats was taken on pre- and post-treatment, that is, initial and final day of post-treatment by digital balance. The blood glucose level of fasted rats was taken pre- and post-treatment, that is, 0, 8th, and 21th day of post-treatment.

At the end of experimental time, all the experimental rats were sacrificed by cervical decapitation. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. Biochemical parameters were studied using automated biochemistry analyzer Hitachi-902.

Estimation of oral glucose tolerance test

Glucose and the oxygen react in the presence of glucose oxidase producing gluconic acid and hydrogen peroxide subsequently.

Estimation of total cholesterol (TC)

TC in serum was estimated using CHOD/PAP methods. Cholesterol is a main component of cell membranes and lipoprotein and it is the precursor for steroid hormones and bile acids synthesizing.

Estimation of triglycerides (TGs)

TGs are the main constituent of vegetable oil, animal fat, LDL, and VLDL and play an important role as transporters of fatty acids as well as serving as an energy source. TGs are broken down into fatty acids and glycerol, after which both can serve as substrates for energy-producing and metabolic pathways. High blood levels of TGs are implicated in atherosclerosis, heart disease, and stroke as well as in pancreatitis. The Triglyceride Quantification Kit provides a sensitive, easy assay to measure triglyceride concentration in variety of samples. In the assay, TGs are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a product which reacts with the probe to generate colorimetric (spectrophotometry at λ =570 nm) and fluorometric (Ex/Em =535 / 587 nm) methods. The kit can detect 2 pmol-10 nmol (or 2~10,000 µM range) of triglyceride in various samples.

Triglyceride Assay Protocol

Standard curve preparation

To perform the colorimetric assay, add 0, 2, 4, 6, 8, and 10 µl of the 1 mM triglyceride standard into wells individually. Adjust volume to 50 µl/well with triglyceride assay buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of triglyceride standard. For the fluorometric assay, dilute the triglyceride standard to 0.01–0.1 mM with the triglyceride assay buffer (detection sensitivity is 10–100-fold higher for a fluorometric than a colorimetric assay). Follow the procedure as the colorimetric assay.

High-density lipoprotein (HDL) cholesterol in serum was estimated using PEG method

Lipoproteins are the proteins, which mainly transport fats in the blood stream. They can be grouped into chylomicrons, very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and HDLs. Chylomicrons and VLDL transport mainly TG, though VLDLs also transport some amount of cholesterol. LDL carries cholesterol to the peripheral tissues where it can be deposited and increase the risk of arteriosclerotic heart and peripheral vascular disease. Hence, high levels of LDL are atherogenic. HDL transports cholesterol from the peripheral tissues to the liver for excretion, hence, HDL has a protective effect. The measurement of total and HDL cholesterol and TGs provides valuable information for the risk assessment of coronary heart diseases.

When the serum is reacted with the polyethylene glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample for cholesterol using the cholesterol (CHOD/PAP) reagent.

Estimation of total protein content

The serum total protein was estimated by modified Biuret method[9] using the total protein test kit (Gupta Pathology, Bhopal).

Estimation of albumin content

The serum albumin was estimated by the method given by Corcoran *et al*., 1977,[10] using albumin test kit (Gupta Pathology, Bhopal).

Estimation of acid phosphatase (ACP) activity

The serum ACP activity was estimated by the method of King *et al*., 1959,[11] using ACP test kit (Gupta Pathology, Bhopal).

Estimation of alkaline phosphatase (ALP) activity

ALP activity was estimated by the method of Kind *et al*., 1954,[12] using ALP test kit (Gupta Pathology, Bhopal).

Estimation of serum glutamate pyruvate transaminase (SGPT)

The enzyme alanine aminotransferase is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations in the study of hepatic diseases. Elevated serum levels are found in hepatitis, cirrhosis, and obstructive jaundice. Levels of ALT are only slightly elevated in patients following a myocardial infarction. UV methods for ALT determination were first developed by Wroblewski and LaDue in 1956.[13] The method was based on the oxidation of NADH by lactate dehydrogenase (LDH). In 1980, the International Federation of Clinical Chemistry recommended a reference procedure for the measurements of ALT based on the Wroblewski and LaDue procedure.

Estimation of serum glutamic oxaloacetic transaminase (SGOT)

SGOT is an enzyme found mainly in heart muscle, liver cells, skeletal muscle, and kidneys. Injury to these tissues results in the release of the enzyme in blood.

Methodology

MOD. IFCC method.

Procedure Assay

Pipette into a clean dry test tube labeled as test (*t*):

Addition sequence	Test (*t*) 37°C
Working reagent	1.0 ml
Sample	0.1 ml

Mix well and read the initial absorbance A0 after 1 min and repeat the absorbance reading after 1, 2, and 3 min. Calculate the mean absorbance change per min.

RESULTS

Body weights of animals in all groups were performed at the onset of study and end of the study. Body weight of animals was significantly ($P < 0.05$) maintained in all treated groups (glibenclamide 500 mg/kg p.o., *L. parviflora* Roxb. 200 and 300 mg/kg/p.o.) [Figure 1].

Blood glucose level of animals in all groups was recorded at 0, 8th, and 21th day. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment glibenclamide 500 mg/kg p.o., *L. parviflora* Roxb. 200 and 300 mg/kg/p.o. (111.00 ± 6.50; 117.00 ± 6.00 and 119.00 ± 5.50) treated group blood glucose level was decrease significantly ($P < 0.05$) at 21st days, respectively [Figures 2-8].

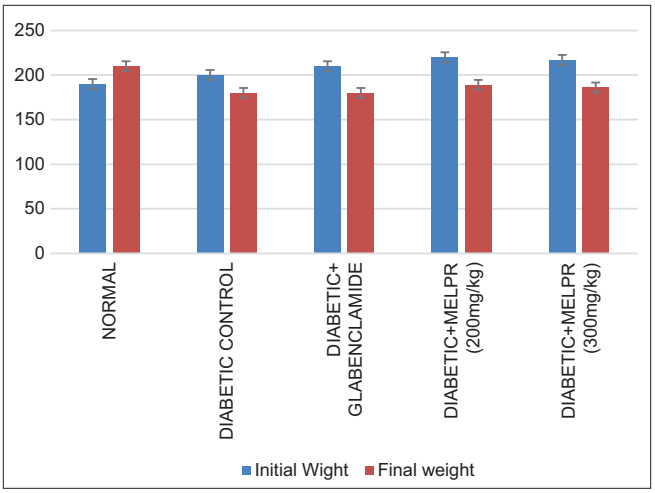

Figure 1: Mean body weight change

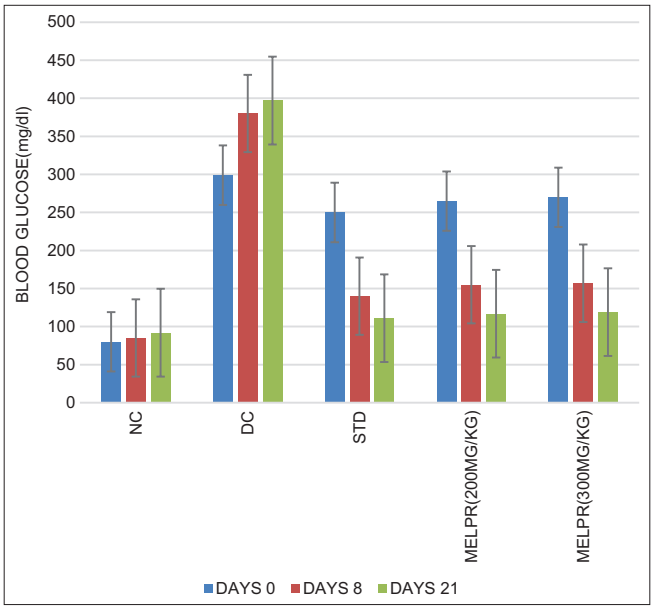

Figure 2: Antidiabetic activity of *Lagerstroemia parviflora* Roxb. on blood glucose level in streptozotocin-induced diabetic rats

DISCUSSION

This antihyperglycemic effect of *L. parviflora* Roxb. (leaves) on the STZ-induced diabetic rats suggests that its main mechanism may not be due to stimulating insulin release from pancreatic cells, but may exert a direct action by promoting glucose utilization by peripheral tissues. We would need much more research work to study the mechanism. Diabetes-induced hyperlipidemia is attributable to the excess mobilization of fat from adipose tissue. In diabetic condition, the activity of enzyme lipoprotein lipase is decreased resulting in increased levels of lipoproteins in the blood. In the present study, a significant

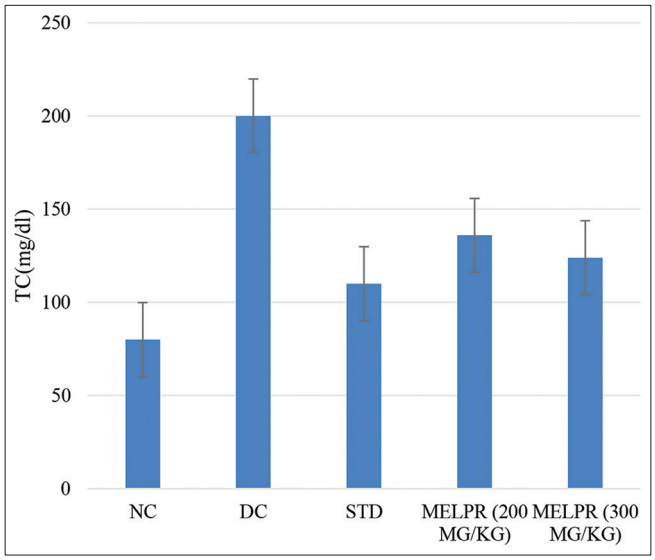

Figure 3: Effect of *Lagerstroemia parviflora* Roxb. on total cholesterol level in streptozotocin-induced diabetic rats

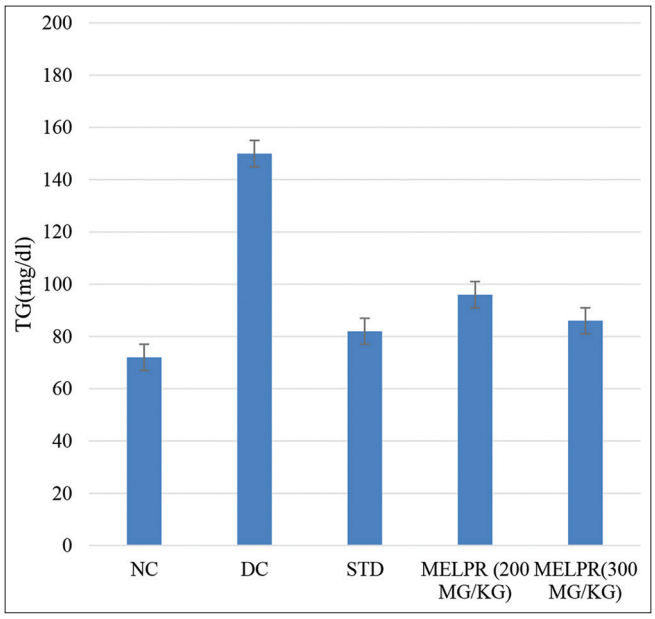

Figure 4: Effect of *Lagerstroemia parviflora* Roxb. on triglyceride level in streptozotocin-induced diabetic rats

decrease in TC, triglyceride, and LDL cholesterol levels was observed after treating the STZ-induced diabetic rats with *L. parviflora* Roxb. (leaves) at 100 and 200 mg/kg body weight leading to a significant increase in the HDL cholesterol levels.

The levels of SGOT and SGPT were significantly altered with the decrease in glucose levels than negative control in diabetic + *Corchorus olitorius* group and overall condition of animals were indicative of classical anti-diabetic activity of *Corchorus olitorius*. Diabetic + *L. parviflora* Roxb. group showed significant glucose levels compare to negative control groups, also *L. parviflora* Roxb. (leaves) outcomes hypoglycemia. SGOT and SGPT levels were increased in the negative control group which indicates might be hepatotoxicity and it

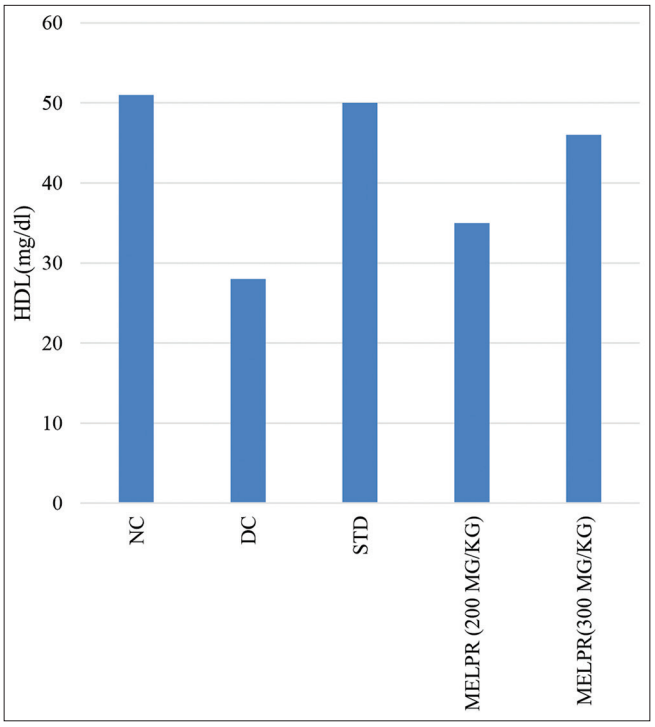

Figure 5: Effect of *Lagerstroemia parviflora* Roxb. on high-density lipoproteins in streptozotocin-induced diabetic rats

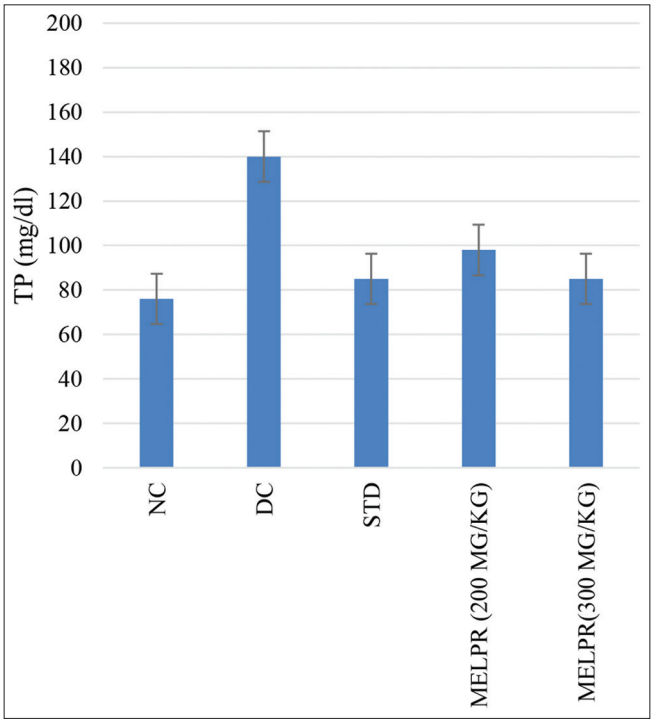

Figure 6: Antidiabetic effect of *Lagerstroemia parviflora* Roxb. on serum lipid profile, that is, total protein level in streptozotocin-induced diabetic rats

was in conjunction with the histopathological observations of minimal to mild hepatocyte degeneration and necrosis with hypertrophy and bile duct proliferation with cytoplasmic clarification (vacuolation) in this study. Diabetic + *L. parviflora* Roxb. (leaves) groups also showed significant

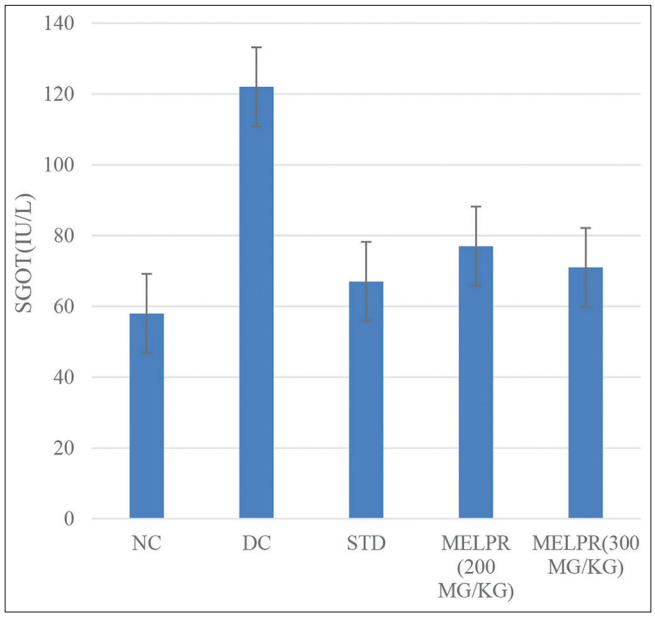

Figure 7: Effect of *Lagerstroemia parviflora* Roxb. on serum glutamic oxaloacetic transaminase in streptozotocin-induced diabetic rats

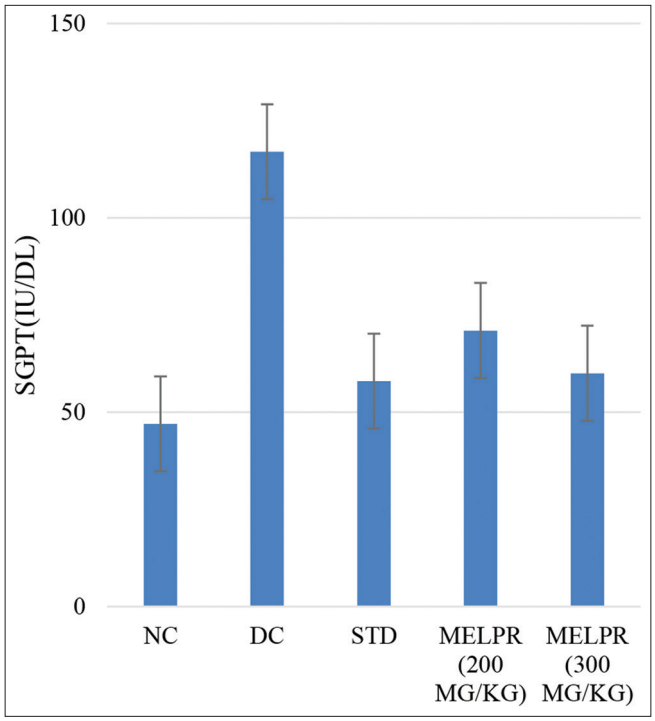

Figure 8: Effect of *Lagerstroemia parviflora* Roxb. on serum glutamate pyruvate transaminase in streptozotocin-induced diabetic rats

($P < 0.05$) as well as histologically minimal hepatocellular hypertrophy and degeneration as compared to negative control in SGOT. The SGOT level was found significant ($P < 0.001$) elevated in diabetic control group.

Moreover, *L. parviflora* Roxb. (leaves) 200 mg/kg (77.50 ± 5.50) treated group SGOT significantly decreased, and *L. parviflora* Roxb. (leaves) 300 mg/kg (71.00 ± 5.00) treated group SGOT also decreased significantly ($P < 0.01$). In 500 mg/kg p.o. metformin (67.00 ± 4.00) treated group, SGOT was significantly decreased ($P < 0.001$), respectively, as compared with control group (122.0 ± 7.00). Although, at the end days of experiment, the serum transaminase such as SGPT level was significantly ($P < 0.001$) elevated in diabetic control group.

L. parviflora Roxb. (leaves) 200 mg/kg (71.00 ± 5.00) treated group SGPT significantly decreased, and *L. parviflora* Roxb. (leaves) 200 mg/kg (60.00 ± 5.00) treated group SGPT also decreased significantly ($P < 0.01$). In 500 mg/kg p.o. metformin (58.00 ± 5.00) treated group, SGPT was significantly decreased ($P < 0.001$), respectively, as compared with control group (117.0 ± 6.00).

The present study showed that biochemical parameters did not show any of the adverse effect of *L. parviflora* Roxb. (leaves) in rats. Liver enzymes such as SGOT and SGPT are considered to be biochemical markers for assessing liver function. *L. parviflora* Roxb. (leaves) significantly reduced the liver enzymes levels in experimental animals show that combined therapy has a hepatoprotective effect. During the experimentation, Wistar rats did not show any mortality or any other adverse effects when the rats fed orally with *L. parviflora* Roxb. (leaves) at the doses of 200 and 300 mg/kg. Hence, *L. parviflora* Roxb. (leaves) has a good safety.

CONCLUSION

It is evident that methanolic extract of *L. parviflora* Roxb. in streptozotocin (STZ)-induced diabetic rats capable of reducing the blood glucose level.

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