Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Evaluation of blood glucose-lowering activity of aqueous extract of bark of Ficus lacor Buch Ham in alloxan-induced diabetes in New Zealand white rabbits

Piyushkumar Mansinh Pargi [1]*, Manish Barvaliya [2], Bhargav Purohit [2]

1. Department of Pharmacology, GMERS Medical College, Godhra, Gujarat, India-389001
2. Department of Pharmacology, Government Medical College, Bhavnagar, Gujarat, India-364001
* Correspondence: Piyushkumar Mansinh Pargi. Department of Pharmacology, GMERS Medical College, Godhra, Gujarat, India-389001.
Tel: +919723416960; Email: drpiyushpargi@gmail.com

Abstract

Background: Although the anti-diabetic effects of various Ficus species have been investigated in animal models, research on the blood glucose-lowering potentials of Ficus lacor Buch Ham bark remains sparse. This study evaluated the blood glucose-lowering potentials of an aqueous extract derived from Ficus labor bark in a diabetic rabbit model.

Methods: Diabetes was induced in rabbits through intravenous administration of alloxan monohydrate (120 mg/kg). 36 rabbits were divided into six groups, each consisting of six animals. Control groups included a non-diabetic control (Distilled water) and a diabetic control (Distilled water). Two experimental groups received Ficus lacor extract at doses of 100 mg/kg (Low-dose) and 200 mg/kg (High-dose) orally for six weeks. Metformin was used as an active control. A non-diabetic group (Extract control) was also administered Ficus lacor extract at 200 mg/kg. Fasting blood sugar (FBS) and post-prandial blood sugar (PP2BS) levels were measured weekly over the 6 weeks. The percentage reduction in blood glucose levels was calculated and compared for each group.

Results: The administration of both low-dose and high-dose Ficus lacor extracts resulted in significant reductions in FBS and PP2BS levels in diabetic rabbits. After six weeks, the low-dose extract group exhibited an average reduction of 38.3% in FBS and 40.5% in PP2BS, whereas the high-dose extract group showed average reductions of 35.3% in FBS and 36.3% in PP2BS.

Conclusion: The aqueous extract of Ficus lacor bark demonstrates substantial glucose-lowering activity, indicating its potential utility as a therapeutic agent in diabetes management.

Article History

Received: 21 June 2023
Received in revised form: 26 September 2023
Accepted: 26 November 2023
Published online: 23 July 2024
DOI: 10.29252/mlj.18.4.1

Keywords

Alloxan
Diabetes mellitus
Ficus
Hyperglycemia
Pre-Clinical

Article Type: Original Article

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood sugar levels either due to insulin deficiency or resistance. Worldwide, 451 million adults have diabetes, and this can increase to as high as 693 million by 2045 (1). In Southeast Asia, 8.5% of the adult population have diabetes, half (49.7%) of which were undiagnosed in 2017 (1). Type 1 DM, due to insulin deficiency, must be treated with regular insulin therapy. Daily insulin injections may lead to poor patient compliance, and it can produce lipodystrophy as a side effect (2). In type 2 DM, sugar homeostasis is affected because of insulin resistance. Treatment of type-2 diabetes includes various oral anti-diabetic drugs due to their long duration of action, such as sulfonylureas, meglitinides, thiazolidinedione, biguanides, and α-glucosidase inhibitors (3). Emerging medications in the management of Type-2 Diabetes Mellitus (T2DM) include incretin mimetics, dipeptidyl peptidase-IV inhibitors, and sodium/glucose co-transporter 2 (SGLT2) inhibitors (3). These drugs have promising effects in controlling blood sugar levels. Still, they have side effects such as frequent hypoglycemia, allergic reactions, lactic acidosis, megaloblastic anemia, weight gain, urinary problems, and gastrointestinal problems, which result in poor patient compliance (4). Moreover, very few drugs have proven effective in the prevention of long-term complications. Hence, research for effective and safer newer anti-diabetic drugs should be continued. WHO also suggests finding new plant-oriented drugs as alternatives to the present drugs (5).

Many herbal drugs have been tested and are being tested for the treatment of DM, and plants of Ficus species are one of them. There are around 850 species in the Moraceae family and Ficus genus. They are found in tropical and subtropical countries such as Southeast Asia, South America, and Australia. Plants of Ficus species have anticancer, anti-inflammatory, antioxidant, and antidiabetic activities (6-8). Various studies have shown that the different Ficus species have the traditional diet-medicine overlay (9). Anti-diabetic properties of various Ficus species have been evaluated in in-vitro and in-vivo studies on crude extracts and isolated compounds (10). They have shown antidiabetic effects by increasing insulin secretion, reducing intestinal glucose absorption, promoting glucose utilization, and increasing insulin secretion (11). As the literature on the antidiabetic effects of Ficus Lacor seems sparse, the present study was planned to determine the efficacy of the aqueous Extract of Ficus lacor bark in diabetic New Zealand White rabbits.

Methods

A. Experimental animals

All experiments were performed following approval from the Institutional Animal Ethics Committee (IAEC), Government Medical College, Bhavnagar, Gujarat, India. New Zealand white rabbits were obtained from the Central Animal House of Government Medical College, Bhavnagar (Registration number 577/GO/Re/S/02/CPCSEA dated 9th August 2016). Thirty-six healthy adult New Zealand white rabbits, female sex, weighing 1.5 to 2.5 kg, were selected for the study. They were housed in stainless steel caging with slotted floors (40 × 60 × 80 cm) and kept under controlled room temperature and humidity (26 ± 3 °C; 40 ± 5 %) in a 12-hour light-dark cycle. They were given a standard laboratory diet and water ad libitum.

B. Chemicals

Alloxan monohydrate powder was procured from Sigma Aldrich, USA. Ficus Lacor Buch Ham extract was procured from Kisalaya Herbals Ltd, Indore, India. Regular insulin (ACTRAPID) manufactured by WOCKHARDT LTD, Aurangabad, India, was procured from government supply from Sir-T Hospital, Bhavnagar. Metformin (FORSON) manufactured by UNISON PHARMACEUTICALS, Ahmedabad, Gujarat, India, was procured from a local medical shop in Bhavnagar, Gujarat, India.

C. Preparation of aqueous extract

Ficus lacor extract dry powder was reconstituted with distilled water in a ratio of 1:1 before administration.

D. Acute oral toxicity study

An acute toxicity assessment was conducted following the guidelines stipulated by the Organization for Economic Cooperation and Development (OECD 423). The selection of the initial dose level followed the protocol, with options comprising four predetermined levels: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight. After dosing, female Swiss albino mice were subjected to individual monitoring for overt behavioral, neurological, autonomic, and toxic manifestations. Observations were recorded at intervals, including at least one assessment within the initial 30 minutes, periodic evaluations over the initial 24 hours (With particular emphasis on the first 4 hours), and daily surveillance extending to 14 days (12). Remarkably, Ficus lacor demonstrated no toxicity, even when administered at the highest 2000 mg/kg dose. Consequently, two doses of Ficus lacor (100 mg/kg and 200 mg/kg) were selected for the current investigation to evaluate their blood glucose-lowering activity.

E. Experimental design

Thirty-six rabbits were randomly divided into the six study groups (n=6 in each group) as per random sequence generated using Rando software (version 1.2). Group 1 (Normal Control) animals received distilled water by oral route. Group 2 (Disease Control) animals received alloxan (120 mg/kg IV) and distilled water by oral route. Group 3 (Active Control) animals received alloxan (120 mg/kg IV) and metformin (62.5 mg/kg) by oral route. Group 4 (Extract Control) animals received aqueous extract of Ficus lacor (200 mg/kg) by oral route. Group 5 (Ficus lacor - low dose) animals received aqueous extract of Ficus lacor (100 mg/kg) by oral route. Group 6 (Ficus lacor - high dose) animals received alloxan (120 mg/kg iv) and aqueous extract of Ficus lacor (200 mg/kg) by oral route. All the treatments were given for six weeks as per the study group. Fasting blood sugar (FBS) and postprandial blood sugar two hours (PP2BS) were monitored weekly for six weeks. 0.5 - 1 ml blood was collected after fasting (12 hours) to measure FBS, and for PP2BS, blood was collected after 2 hours of feeding.

F. Experimental induction of diabetes

A freshly prepared 120 mg/kg solution of alloxan monohydrate (Sigma Aldrich, USA) in distilled water was administered intravenously through a marginal ear vein to induce diabetes in rabbits. After 72 hours of alloxan injection, random blood sugar (RBS) was measured using a glucometer. Rabbits having RBS > 200 mg/dl were considered diabetic and were included in the study. Those rabbits that did not achieve RBS > 200 mg/dl after 72 hours were injected with a second dose of alloxan monohydrate (60 mg/kg). Even after 72 hours of a second dose of alloxan, if the RBS level was not increased > 200 mg/dl, those animals were excluded from the study. RBS-level monitoring was performed daily in the evening for animals included in the study. The rabbits were given subcutaneous insulin as per their RBS level for maintaining blood sugar levels between 200-400 mg/dl and reducing mortality due to uncontrolled sugar levels. 2 IU/kg, 3 IU/kg, and 4 IU/kg insulin were given to the rabbits if RBS level was found 400-500, 500-600, and > 600 mg/dl, respectively (13).

G. Statistical analysis

Primary outcome variables such as FBS and PP2BS were expressed as mean ± standard deviation (SD). Comparisons of FBS and PP2BS were made within the study groups for various time points using repeated variance analysis measures followed by a posthoc test. In contrast, comparisons across the study groups were done by one-way analysis of variance followed by the Tukey-Kramer post-hoc test. The percentage of blood sugar reduction at weeks one to six was calculated by subtracting the sugar level of the respective time point from the baseline sugar level and dividing that by the baseline sugar level.

The percentage of blood sugar level reduction was expressed in mean ± standard deviation (SD) and compared between the groups using the one-way analysis of variance followed by the Tukey- Kramer posthoc test. Graphpad Instat3 (Demo version) was used for the data analysis, and the statistical significance was set for $P < 0.05$.

Results

Thirty rabbits developed persistent hyperglycemia (Blood glucose > 200 mg/dl) after the single dose of alloxan, and six required a second dose.

H. Effect on blood sugar levels

The administration of alloxan significantly increased the FBS and PP2BS levels of disease-controlled rabbits compared to those of normal-controlled rabbits. The study groups had a significant difference in baseline FBS and PP2BS levels. However, in the disease control group, FBS and PP2BS were elevated throughout the study. Administration of 100 mg/kg and 200 mg/kg *Ficus lacor* extract caused a significant decrease in FBS and PP2BS compared to the disease control group. Comparison of FBS and PP2BS levels between various study groups at different time points are shown in Tables 1 and 2.

I. Percentage reduction in blood sugar level

Administration of low dose (100 mg/kg) *Ficus lacor* extract caused a significant reduction in the percentage of FBS levels compared to the disease control group from the fourth week, whereas high dose (200 mg/kg) *Ficus lacor* extract and metformin decreased FBS from the 5th week. A similar observation was found for PP2BS. At the end of the 6th week, low dose *Ficus lacor* caused an average 38.3 and 40.5 % reduction in FBS and PP2BS, respectively, whereas high dose *Ficus lacor* caused an average 35.3 and 36.3 % reduction in FBS and PP2BS, respectively. Reduction trends of FBS and PP2BS are shown in Figures 1 and 2.

Table 1. Comparisons of fasting blood sugar (mg/dl) at various study time points level

Group	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	p-value
NC	99.6 ± 7.1	94±6.1	105 ± 4	104.1 ± 5.1	105.6 ± 3.6	103 ± 4.5	97.5 ± 5.7	0.0062
DC	507.8 ± 83.1 #	474.1 ± 133.9 #	481.5 ± 117.9 #	518.6 ± 113 #	498 ± 130.4 #	501.5 ± 139.8 #	467.3 ± 105.1 #	0.5217
AC	437.6 ± 109.1*	345.6 ± 115.7*	370.6 ± 87	335.8 ± 124.2	284.5 ± 60.1$	266.6 ± 60.5$	238.1 ± 54.1$	< 0.0001
EC	136.1 ± 25.5	117.8 ± 12	104.8 ± 2.9$	108.5 ± 8.7$	110.8 ± 9.5$	103.1 ± 4.7$	98.3 ± 5.4$	0.0001
LDE	328.8 ± 78.8*	244.5 ± 98.8	243.3 ± 72.9	219.8 ± 75	207.5 ± 73.3$	206.3 ± 68.3$	203.8 ± 73$	0.0013
HDE	317.5 ± 79.9	279.3 ± 132.5	257.3 ± 104.8	298.1 ± 141.4	256.1 ± 140.8	214.8 ± 122.3	207.8 ± 82.9	0.0441
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-

- P-value < 0.05 as compared to NC; * - P-value < 0.05 as compared to DC, $- P-value < 0.05 as compared to Baseline. NC - Normal Control group, DC- Disease Control group, AC -Active Control group, EC - Extract Control group, LDE - Low Dose Extract group, HDE - High Dose Extract group.

Table 2. Comparisons of PP2BS (mg/dl) at various study time points

Group	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	p- value
NC	111.5 ± 4.6	107.8 ± 5.8	101.5 ± 9.1	96.3 ± 10.4	100.8 ± 9.6	88.8 ± 5.1	102.8 ± 12.8	0.0047
DC	470.8 ± 60.4 #	441.1 ± 106.5 #	452.6 ± 99.8 #	472 ± 92.3#	468.8 ± 109.2#	461.1 ± 114.3#	441.1 ± 82#	0.7121
AC	388.5 ± 99.4	288.1 ± 119.2	323.6 ± 92.5	297.5 ± 125.3	254.1 ± 64	238.6 ± 65.6$	219 ± 64$	0.0016
EC	128 ± 28.3	111.8 ± 17.6	101.5 ± 9.1$	96.3 ± 10.4$	98.6 ± 6.5$	88.8 ± 5.1$	102.8 ± 12.8	0.0019
LDE	302.1 ± 81.2	215.5 ± 83.8	213.6 ± 62.9*	200.8 ± 71.8	187.1 ± 69	182.6 ± 63.3$	180.6 ± 69$	0.0043
HDE	295.6 ± 68.2	272.8 ± 142.7	240.5 ± 97.9#*	281.6 ± 132.6	241.1 ± 129.2	196.1 ± 112.7	191.3 ± 82	0.0364
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-

- P- value < 0.05 as compared to NC; * - P-value < 0.05 as compared to DC, $- P-value < 0.05 as compared to Baseline. NC - Normal Control group, DC- Disease Control group, AC -Active Control group, EC - Extract Control group, LDE - Low Dose Extract group, HDE - High Dose Extract group.

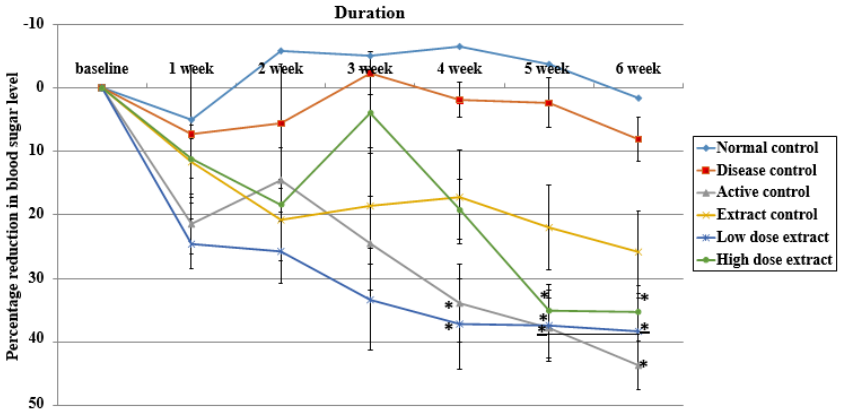

Figure 1. Percentage reduction in FBS (fasting blood sugar) level in Ficus lacor Buch.-Ham.

*P < 0.05 as compared to disease control

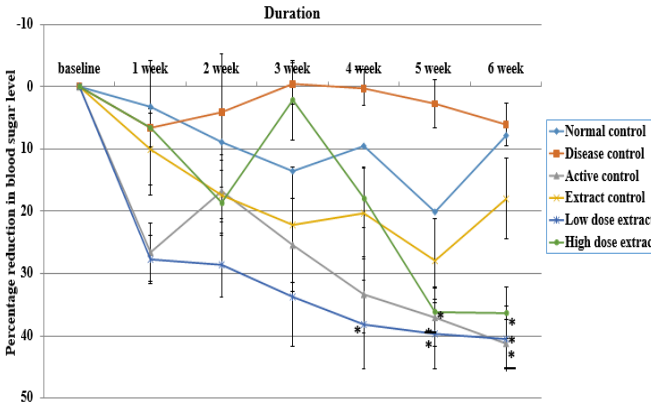

Figure 2. Percentage reduction in PP2BS (post prandial blood sugar) level in Ficus lacor Buch.-Ham. *P < 0.05 as compared to disease control

Discussion

We evaluated the blood glucose-lowering effect of Ficus lacor in the animal model of alloxan-induced diabetic rabbits. Alloxan destroys β- cells in the pancreas and reduces insulin secretion, leading to persistent hyperglycemia (14). Treatment with Ficus lacor significantly reduced FBS and PP2BS compared to disease control. At the end of the 6th week, low dose Ficus lacor caused 38.3 ± 11.8 and 40.4 ± 11.4 % reduction in FBS and PP2BS levels, respectively (Figures 1 and 2). At the end of the 6th week, high dose Ficus lacor caused 35.2 ± 12.5% and 36.3 ± 14.5 % reduction in FBS and PP2BS levels, respectively (Figures 1 and 2). These reductions were significant as compared to the disease control group. Thus, Ficus lacor showed blood glucose-lowering effects in diabetic rabbits. However, based on our findings, a low dose (100 mg/kg) showed an early and more significant reduction in FBS and PP2BS levels compared to a high dose (200 mg/kg). The other Ficus species (Such as Ficus racemosa and Ficus benghalensis) have shown anti-diabetic activity by enhancing insulin secretion and sensitivity, increasing glycogen synthesis, decreasing gluconeogenesis, and decreasing glucose absorption by inhibiting α glucosidase and α amylase (6). In a study conducted by Pandit et al. in 2010, it was demonstrated that Ficus religiosa, administered at three distinct doses (25, 50, and 100 mg/kg), elicited reductions in blood sugar levels amounting to 28.56%, 49.65%, and 48.58%, respectively, by day 21 (15). Therefore, compared to Ficus religiosa, Ficus lacor may exhibit a comparatively weak blood glucose-lowering effect when administered at 100 mg/kg. We used metformin as an active control that caused 43.6 ± 13.2% and 41.2 ± 17.7% reductions in FBS and PP2BS, respectively (Figure 1 and 2). Metformin caused a more prominent glucose-lowering effect than both low doses (100 mg/kg) and high doses (200 mg/kg) of Ficus lacor. Metformin enhances insulin sensitivity, promotes insulin binding to its receptors, inhibits gluconeogenesis, and reduces glucose absorption.

Ficus lacor in normal animals also reduced FBS and PP2BS by 25.8 ± 13% and 18 ± 10.8%, respectively. This also suggests the glucose-lowering activity of Ficus lacor in normoglycemic rabbits and the possible chances of hypoglycemia as a side effect. Diabetes is associated with oxidative damage, resulting in various complications, and anti-oxidant activities can help in preventing diabetic complications (6). Ficus lacor is a potential source of antioxidants and can be evaluated further to avoid diabetic complications (16). Gupta S et al. (2008) examined the compositions of Ficus lacor and found that it contains the antioxidants such as β-carotene and Vitamin C, high fiber such as acid, and neutral detergent fiber, which may have a significant role in preventing diabetes (17). The phytochemical evaluation of Ficus lacor has shown that it contains carbohydrates, alkaloids, saponins, flavonoids, and sterols. Flavonoids and sterols may contribute to antidiabetic effects (18). β -Sterols enhance insulin release and help in controlling blood sugar (19). In contrast, flavonoids have different actions for blood glucose-lowering effects, which include the inhibition of intestinal glucose absorption by inhibiting α -glucosidase and improvement in glucose tolerance (20). The presence of flavonoids and sterols in Ficus lacor might be responsible for the anti-hyperglycaemic effect. In the present study, we could not determine the mechanism of action of Ficus lacor for blood sugar reduction, and we also could not evaluate the effect on HbA1c. These were the limitations of this study. Further studies should be carried out for the evaluation of the antidiabetic mechanisms of Ficus lacor.

Conclusion

Aqueous extract of Ficus lacor demonstrated a significant reduction in FBS and PP2BS at the end of 6 weeks as compared to the disease control group suggesting its hypoglycaemic effects. Mechanisms of action for hypoglycaemic effects can be explored by doing further studies.

Acknowledgement

We are sincerely thankful to ICMR (Indian Council of Medical Research), New Delhi, for providing a dissertation grant for this project. Grant No.3/2/June-2017/PG- Thesis-HRD (19).

Funding sources

Not applicable.

Ethical statement

The Institutional Animal Ethics Committee (IAEC) of Government Medical College, Bhavnagar, Gujarat, India, approved this study (Approval no. 58/2017, approval date 28/10/2017). During this study, the research adhered to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

Conflicts of interest

The authors state no conflict of interest.

Author contributions

All the authors were involved in study design, execution, data analysis, and report writing. All authors accept the responsibility for the content of this manuscript.

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How to Cite:

Pargi PM, Barvaliya M, Purohit B. Evaluation of blood glucose-lowering activity of aqueous extract of bark of Ficus lacor Buch Ham in alloxan-induced diabetes in New Zealand white rabbits. *Med Lab J.* 2024;18(4):1-4.