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Original Article

ANTIHYPERGLYCAEMIC EFFECT OF *FICUS DALHOUSIAE* MIQ LEAF ETHANOLIC EXTRACT IN ALLOXAN–INDUCED DIABETIC RATS

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ABSTRACT

Objective: *Ficus dalhousiae* Miq. Has been documented for a wide range of uses in Ayurvedic and Unani medicine. The aim of this study was to evaluate the anti-hyperglycaemic effect of FDLEE in alloxan induced diabetic rats.

Methods: Plant material was collected from Tirupati A. P, during the month of March 2013 the leaves were wiped carefully to make them free from dust and foreign material and dried under shade at room temperature. After seven days, the leaves were powdered and passed through a sieve. The powder was weighed (500 gm) and was extracted by maceration process and the solvent was evaporated in a rotavapor at 40º- 50º C under reduced pressure. The total yield of the extract was 16.5%. Phytochemical screening was carried out for the detection of alkaloids, flavanoids, glycosides, saponins, sterols and tannins by simple qualitative methods. Diabetes mellitus was induced by single i. p injection of freshly prepared solution of Alloxan monohydrate at a dose of 150mg/kg b. w. The animals were kept under observation for 48hr; Blood glucose was measured by glucometer. The rats with blood glucose levels above 250 mg/dl were selected for the experimental studies. FDLEE (100, 200 & 400mg/kg, b. w) was administered orally once a day for a period of 10 days. Body weight and blood glucose levels were determined on different experimental days.

Results: Significant decrease in body weight and increase in blood glucose and lipid profile were observed in diabetic rats. The administration of FDLEE and glibenclamide daily for 10 days reversed body weights and blood glucose significantly.

Conclusion: FDLEE exhibited anti-hyperglycaemic and anti-hyperlipidaemic effects in diabetic rats which supports its use as an adjunct in treatment of diabetes.

Keywords: *Ficus dalhousiae* leaf ethanolic extract, Body weight**,** Intraperitonial.

INTRODUCTION

Diabetes mellitus is a multi-factorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities, raised metabolic rate, defect in reactive oxygen species, scavenging enzymes and altered metabolism of major food substances. Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders [1]. Diabetes has become one of the devastating diseases afflicting the health of many people in recent times and has accounted for a high proportion of health problems worldwide. It is becoming the third major disease of mankind, after cancer and cardiovascular diseases because of its high prevalence, morbidity and mortality [2]. In modern medicine, no effective treatment is available to treat diabetes mellitus [3]. Diabetes Mellitus has assumed epidemic proportion in many countries in the world. There are estimated 143 million people in the world with diabetes, which is almost 5 times more than the estimation of ten years ago. This number will probably be doubled by 2030 [4]. Many oral hypoglycaemic agents such as sulfonylureas and biguanides are available along with insulin for the treatment of diabetes, but these agents have significant side effects and some are ineffective in chronic diabetic patients [5]. Despite of the great effort made in the understanding and management of diabetes, the disease and disease related complications are increasingly unabated [6]. Therefore, there is a great need for a search for an acceptable, cheap and safe blood sugar lowering oral hypoglycaemic agent that would be effective in treatment of diabetes and devoid of serious side effects,interest has thus been shifted to use of alternative medicine[7]. Medicinal plants are continued to be a powerful source for new drugs. Now contributing about 90 % of the newly discovered pharmaceuticals. Traditional medicines provide better health coverage for 80 % of the world population, especially in the developing countries [8]. *Ficus dalhousiae* is a small tree belonging to family Moraceae [9], which is reported to possess the following medicinal uses: Fruit is used as cardio tonic. Leaves and bark are used in affections of the liver and skin diseases [10]. The other species of Ficus genus like *Ficus racemosa* which contain flavonoids as active antidiabetic principles have been reported earlier in literature [11]. The goal of this present study was to evaluate antihyperglycemic activity of FDLEE in alloxan induced diabetic rats based on the preliminary phytochemical report which showed the presence of flavonoids.

MATERIAL AND METHODS

Plant material was collected from Tirupati A. P, during the month of March 2013. Authentication of plant material was previously done by Department of Botany, Osmania University, Hyderabad -500 007. India. The plant was given Voucher No 0949.

Chemicals

All the chemicals required for preliminary phytochemical screening and pharmacological evaluation were of analytical grade. The solvents were purchased from standard trading company, Tilak road. Hyderabad (Sd fine chemicals limited). Alloxan Monohydrate (Spectrochem Pvt. Ltd) was used for inducing diabetes. Dextrose from (Emkay Labs,India), Anaesthetic ether from Ozone International,Mumbai were procured. Accu-chek Active Glucometer. Roche Diagnostic Corporation, Germany and blood gluco-strips were from (Roche Diagnostic Pvt. Ltd. Mumbai, India)

Method of Preparation of plant extract

Leaves of *Ficus dalhousiae* were wiped carefully to make them free from dust and foreign material and dried under shade at room temperature. After seven days of drying, the leaves were powdered by grinding and passed through a sieve. The powdered leaves were stored in air tight container for further use. The powder was weighed (500 gm) and was soaked in 1500 mL of 95 % ethanol overnight, after filtration the residue was again suspended in equal

volume of 95% ethanol for 48 hr and filtered again. The above filtrates were mixed and the solvent was evaporated in a rotavapor at 40º- 50º C under reduced pressure [12]. The dried extract was kept in air-tight container for further use. The total yield of the extract was 16.5%

Phytochemical Screening

Phytochemical screening was carried out for the detection of tannins, alkaloids, flavonoids, glycosides, sterols and saponins by simple qualitative methods [13].

Induction of diabetes in experimental rats

Alloxan was weighed individually for each animal according to body weight and then solubilised with 0.2 ml sterile saline (154 mM NaCl) just prior to injection. Diabetes mellitus was induced by single i. p injection of freshly prepared solution of Alloxan monohydrate at a dose of 150mg/kg b. w. [14]. After 1 hr. of alloxan administration, the animals were given feed ad libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycaemic phase. The animals were kept under observation for 48hr; blood glucose was measured by glucometer. The rats with blood glucose levels above 250 mg/dl were selected for the experimental studies.

Experimental design

36 matured albino wistar rats weighing about 150-200 gm were procured from Sainath Agencies, Bapujinagar, Musheerabad, Hyd-48 Reg NO.282. The animals were stabilized for a week than were acclimatized to standard laboratory conditions. They were fed with commercial pellets and were given free access to water ad libitum throughout the course of the study the experimental handling and care of laboratory animals were as per the Institutional Animal Ethical Committee guidelines bearing registration No. 1534/PO/a/11CPCSEA.

Rats were randomly divided into six groups of six animals each. The treatment schedule was as follows

1. Normal control Group- (Normal saline 1 ml/kg)
2. Diabetic Control Group- (Alloxan monohydrate 120 mg/kg b. w; i. p)
3. Standard group- (Glibenclamide 10 mg/kg, b. w; p. o.)
4. Test 1 (FDLEE) group-100 mg/kg (b. w; p. o.)
5. Test 2 (FDLEE) groups- (200 mg/kg b. w; p. o.)
6. Test 3 (FDLEE) group- (400 mg/kg b. w; p. o.)

After 5 days of Alloxan injection, the hyperglycaemic rats (glucose levels>250 mg/dl) were separated and included in the anti-diabetic study. The treatment (p. o.) was started from the same day except normal control and diabetic groups for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated on 4th, 7th, 10th day of the treatment. On the 10th day, blood samples were collected from overnight fasted rats from retro orbital sinus by using capillary tubes into vials containing sodium fluoride and sodium oxalate as anticoagulant mixture. The serum was separated by centrifuge at 2000 rpm for 2 minutes. For the biochemical estimation[15]. The animals were sacrificed after blood collection under mild ether anaesthesia, & their pancreas were removed, for the histopathological studies.

Method of Determination of Acute Toxicity

Acute oral toxicity study was performed as per OECD-423 guidelines category IV (acute toxic class method,). Albino mice (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were kept fasting for 4 hrs with free access to water only.

The plant extract was administered orally with maximum dose of 2000 mg /kg body weight by gastric intubation. The mortality was observed for three days. If mortality was observed in 2 out of 3 animals or 3 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 3000 mg/kg of body weight [16].

Determination of Serum Glucose

The serum concentrations of glucose were determined by Glucose-oxidase method[17], Blood cholesterol was determined by diagnostic kit which was available at veterinary and Biological Research Institute, Shantinagar, Vijayanagar Colony, Hyderabad.

Procedure of Histopathological Studies

The histopathological studies were carried out at Veterinary and Biological Research Institute, Shantinagar, Vijayanagar Colony, Hyderabad Pancreas of rats were taken immediately from stomach, fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 µm thick) were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation, including cell necrosis, fatty change and improvement of the β-cells of islets of langerhans.

Statistical analysis

The results of the biochemical estimations were reported as Mean ± SEM and were analysed by one way analysis of variance (ANOVA) followed by Dunnett's 't' test. 'P' value <0.05 was considered as statistically significant.

RESULTS

Results of Phytochemical Screening

The phytochemical screening of ethanolic extract of *Ficus dalhousiae* leaves showed the presence of Alkaloids, Glycosides, Tannins, Flavonoids and Reducing Sugars. Tests were negative for Sterols and Saponins (Table 1)

Effect of FDLEE on body weight in experimental groups

The changes in body weights before and after treatment of the test drug in Alloxan induced diabetic rats is given in table. In the diabetic control group the body weights were decreased than those in normal rats. Diabetic rats treated with extract showed a significant increase in body weights ($P<0.05$) as shown in table 2.

Effect of FDLEE on blood glucose in experimental groups

The Alloxan administration in experimental animals resulted in significant ($P<0.05$) rise in blood glucose levels. .In diabetic rats treated with extract showed significant dose dependent lowering of blood glucose levels (Table 3)

Effect of FDLEE on Serum cholesterol in experimental groups

The cholesterol levels decreased in the normal control group and increased in diabetic control group. There was also decrease in the cholesterol levels in the standard and test groups when compared with the diabetic control group.(Table 4)

Result of Histopathological studies (fig 4)

The photomicrographs of normal control group rats showed normal acini, and normal cellular population in the islets of Langerhans in pancreas as shown in (slide 1). Massive cell damage, extensive destruction of cell lining, inflammatory cells, and β-cell damage were seen in diabetic control group (slide 2). There was enlargement of β-cells, increase in vascular spaces with hyperplasia in standard group, i. e. Glibenclamide (slide 3).

The histopathological results of pancreas with FDLEE 100 mg/kg showed exocrine and endocrine tissue at most places intact with few areas showing presence of fatty tissue. The slight damage to the cell lining, slight damage to vasculature and haemorrhages with necrosis were also seen.

The microscopic changes showed significant improvement in the development of islet of Langerhans (slide 4). The microscopic changes in pancreas with FDLEE 200 mg/kg showed haemorrhage with necrosis, damage to the vasculature, normal cell population, slight β-cell enlargement.(slide 5) In the test group FDLEE 400 mg/kg there was increase in vascular spaces, rarely seen mild necrosis and significant β-cell recovery after administration of the extract. (Slide 6).

DISCUSSION

The present study has revealed the anti-diabetic effects of the ethanolic leaf extract of *Ficus dalhousiae* in Alloxan induced diabetic rats. The investigations indicate the efficacy of the ethanolic leaf extract of *Ficus dalhousiae* in the maintenance of blood glucose levels in Alloxan-induced diabetic rats. Further, induction of diabetes with Alloxan is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins. There was significant increase in body weight in extract treated diabetic rats with respect to diabetic control. Diabetes is often linked with abnormal lipid metabolism and is considered as a major factor in the development of atherosclerosis and cardiovascular complication.

The World Health Organisation expert committee recommends the importance of investigation and exploration of hypoglycaemic agents of plant origin because plants used in the traditional medicine have got fewer side effects than synthetic drugs. It has been clearly demonstrated that FDLEE has a potential efficacy in lowering glucose metabolism and lipid metabolism.

Table 1: Phytochemical Screening Report – FDLEE

TEST	RESULTS
Glycosides	+
Alkaloids	+
Tannins	+
Saponins	-
Flavonoids	+
Anthraquinones	+
Sterols	–
Reducing Sugars	+

(+) Present and (-) Absent

Table 2: Effect on body weight of animals in different experimental groups

Treatment	Dose (mg/kg)	Body Weight (g)			
		Day 1	Day 4	Day 7	Day 10
rmal control	10 ml	161.16 ±2.97	160.5±2.07	163±2.95	160.66±2.31
Diabetic control	120	189.5±3.54	165.33±3.41	160±4.11	154.5±0.92
FDLEE	100	188.33±3.12	169.16±4.63	155.66±1.58	152±0.85
FDLEE	200	181±4.25	170±2.62	163.33±1.85	159±2.68
FDLEE	400	183±5.02	176.83±0.90*	173±1.55**	167.66±2.04**
Glibenclamide	10	186.66±2.96	179.83±3.16**	176.33±2.40**	173.33±1.25**

Table 3: Changes in Blood glucose levels in different experimental groups

Groups	Treatment	Basal value	4th day	7th day	10th day
I	Normal control Normal Saline(1 mL/kg)	92.5± 0.55	83± 1.96**	90± 1.55**	90.5± 1.34**
II	Diabetic control Alloxan(120 mg/kg)	294.5± 7.38	294.5± 9.11	303± 3.07	309± 7.14
III	Glibenclamide(10mg/kg)	270± 2.67	239.5± 2.30 **	213.5± 1.97**	199.5±16.7**
IV	FDLEE(100 mg/kg)	290± 4.83	279.5±3.69	278± 3.88*	241.5±1.90**
V	FDLEE(200 mg/kg)	284± 4.16	277± 3.59**	256.5± 3.47**	237.5±1.62**
VI	FDLEE(400 mg/kg)	281.5± 4.26	264.5± 3.18**	241.5± 2.81**	231.5±1.80**

Table 4: Changes in blood cholesterol levels in the experimental groups

S. No	Normal Control	Diabetic Control	Standard 120 mg	FDLEE 100mg	FDLEE 200mg	FDLEE 400mg
1	106	178.99	121.81	165.25	155.28	144.9
2	102	175.24	122.34	162.34	152.44	142.32
3	104	176.45	125.45	168.44	162.42	148.55
4	108	165.55	123.66	162.34	158.24	135.55
5	105	168.56	124.33	166.55	152.66	139.54
6	106	174.99	122.44	155.55	151.52	138.98
Mean ± SEM	105.166±0.83	173.296±2.09	123.336±0.56**	163.41±1.84**	155.426±17**	141.64±1.89**

In addition, alloxan injection caused diabetes, which may be due to destruction of beta-cells of the islets of Langerhans. The effects were dose dependent and the reduction of blood glucose may be either due to the increase in the level of plasma insulin in diabetic rats, which may influence the stimulation of pancreatic insulin secretion from beta-cells in islets of Langerhans, or due to the enhanced transport of blood glucose to peripheral tissues. Serum cholesterol levels were decreased significantly by glibenclamide and all the extracts of FDLEE during10 days of treatment. In diabetes hyperglycaemia is accompanied with dyslipidaemia [18] characterized by an increase in TC, LDL, VLDL, and TG and fall in HDL. The Serum cholesterol levels were reversed towards normal after treatment with FDLEE. Diabetes is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [19]. Previous studies have demonstrated that diabetes exhibits enhanced oxidative stress and high reactive oxygen species (ROS) in

pancreatic islets due to persistent and chronic hyperglycaemia, thereby depletes the activity of anti-oxidative defence system, and thus promotes free radical generation [18]. Alloxan is widely employed to induce diabetes mellitus in experimental animals due to the fact that it causes severe necrosis of pancreatic β-cells with consequent lack of insulin secretion. Increased oxidative stress induced by alloxan is regarded as the possible mechanism of its hyperglycaemic action [20] Beneficial roles such as correction of altered carbohydrate metabolism, maintenance of integrity and function of β-cells, insulin-secreting activity, enhancement of glucose uptake and utilization and antioxidant properties present in the traditional medicinal plants and their constituents offer exciting opportunity to develop them into novel therapeutics [21]

The possible mechanism through which FDLEE exert anti-hyperlipidaemic effect might be due to changed activity of cholesterol biosynthesis enzymes or due to changed level of lipolysis which are under the control of insulin [22]. Blood glucose level and body weight have been commonly measured to monitor the glycaemic control mechanism. In the present study, diabetic rats had lower body weight, high blood sugar levels as compared to normal rats. However, orally administered FDLEE significantly increased the body weight and decreased the blood glucose level.

This could be due to potentiation of the effect of insulin by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of FDLEE in alloxan induced diabetic rats may also be due to enhanced glucose utilization by peripheral tissues [23]. Hyperglycaemia is a main cause for elevated free radical levels followed by production of ROS, which can lead to increased lipid peroxidation and altered antioxidant defence and further leads to impairment of glucose metabolism in biological system [24]. Hypercholesterolemia has been reported earlier in alloxan-induced diabetic rats [25]. The result of this study reveals that the dose of 400 mg/kg lowered serum cholesterol significantly.

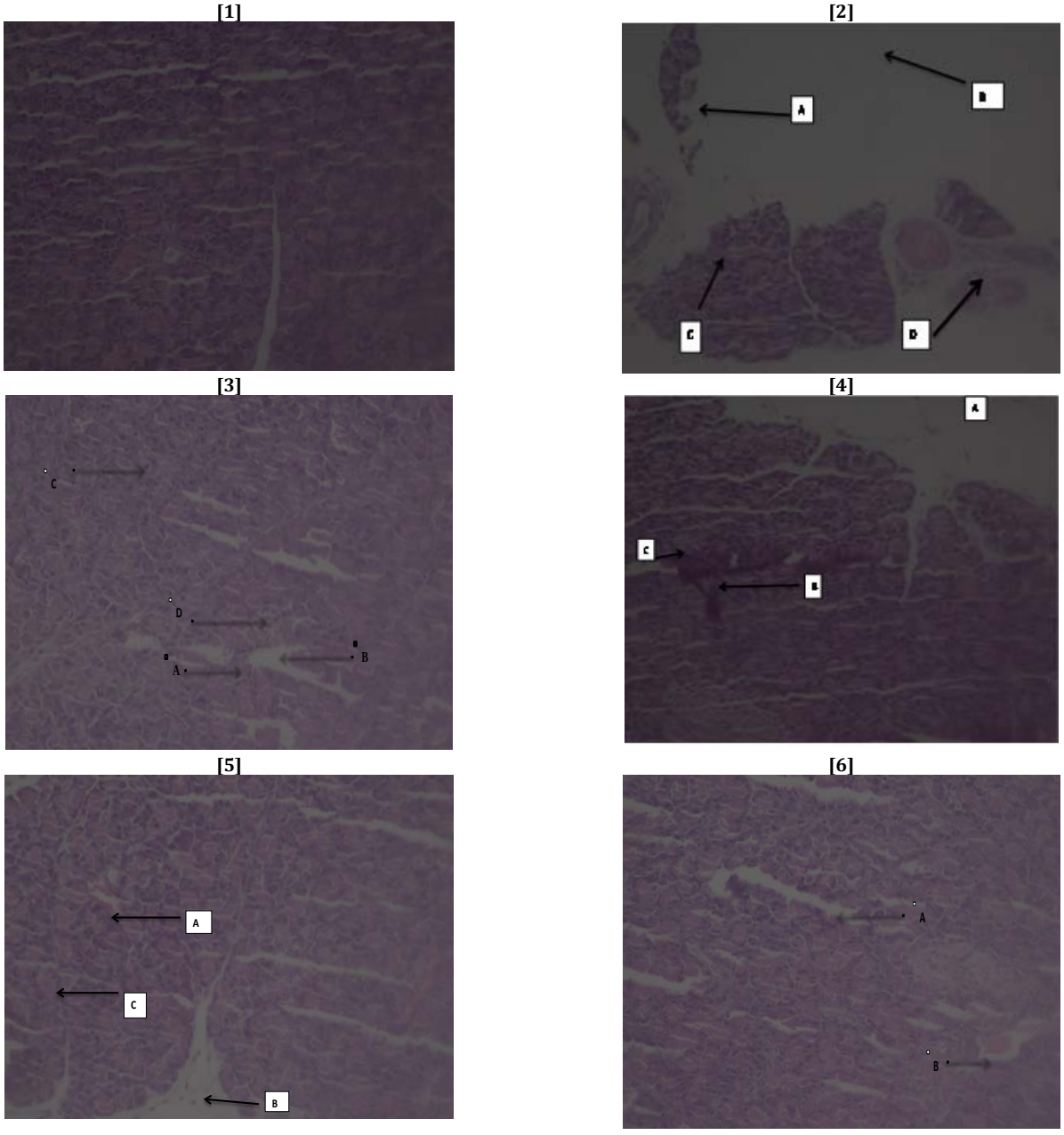

Fig 4: Slides of pancreas of different treated groups: (1- Normal Control; 2-Diabetic control; 3-Standard; 4-Test I; 5-Test II; 6-Test III) A- Massive cell damage; B- extensive destruction of cell lining; C- inflammatory cells; D- β-cell damage

Bioactive principles isolated from plants like Phenols [26], Flavonoids [26,27], triterpenoids alkaloids [28,29] and carbohydrates [30] have shown antihyperglycemic effect[31]. The phytochemical investigation revealed the presence of flavonoids, tannins, alkaloids, in ethanolic extract. Presence of wide range of constituents like flavonoids and tannins indicated the good anti-diabetic efficacy of this plant. Presence of flavonoids is well known for their anti-diabetic activity by different mechanisms. Flavonoids act by one or more of the following mechanisms [32]. as insulin secretagogues or insulinomimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, &regulate the activity &or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway [33].

CONCLUSION

From the results obtained in the current investigation, it may be concluded that ethanolic leaf extract of *Ficus dalhousiae* possess significant anti-diabetic activity and it might be of help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of anti-diabetic drugs. Alloxan which is a possible β cytotoxin causes a massive destruction of β cells of the islets of Langerhans resulting in reduced synthesis and release of insulin. The functioning of insulin system is suppressed leading to elevated blood glucose level. These conditions may also be responsible for the death of the animals. FDLEE exhibited potent antidiabetic effect in alloxan–induced diabetic rats and reduced the mortality rate significantly. Further research can be done for developing a formulation of *Ficus dalhousiae* leaves and to conduct its dose studies.

ABBREVIATIONS

FDLEE- *Ficus dalhousiae* leaf ethanolic extract

B. W: Body weight

I. P: Intraperitonial

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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