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ORIGINAL ARTICLE

Alpha Glucosidase Inhibiting Activity and *in vivo* Antidiabetic Activity of *Fraxinus floribunda* Bark in Streptozotocin-Induced Diabetic Rats

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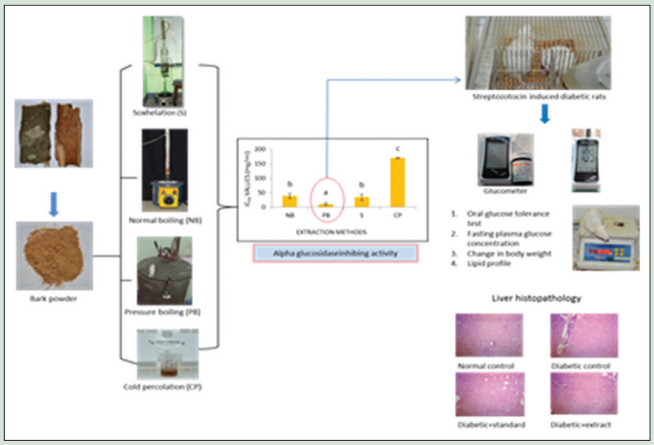
ABSTRACT

Background: Diabetes mellitus is a serious health problem being the third largest cause of death worldwide. Natural sources of antidiabetic agents are of high demand due to side effects of modern drugs. Bark of *Fraxinus floribunda* (FF) is conventionally used in Sikkim to treat diabetes, but there is not a single documented report on the same. **Objective:** The aim of this study is to evaluate *in vitro* and *in vivo* antidiabetic activity of FF bark. **Materials and Methods:** FF bark was extracted through four methods, namely normal boiling, pressure boiling (PB), soxhlet, and cold percolation to be subjected to α-glucosidase inhibiting assay. The extract showing the highest *in vitro* antidiabetic activity was selected for *in vivo* antidiabetic activity. **Results:** Extract from PB showed the highest antidiabetic activity (10.25 ± 4.56 mg/ml FWT); thus, it was selected for antidiabetic property in animal model. The extracts (200 and 400 mg/kg) significantly ($P < 0.05$) reduced plasma glucose concentration in streptozotocin-induced diabetic rats. Glibenclamide (0.50 mg/kg) was used as standard. Decrease in bodyweight during diabetes was significantly controlled by the extract which was comparable with the standard at the same concentration. Changes in lipid profile (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein) of the diabetic rats were also maintained almost to the level of normal rats by the extracts. Histopathology of liver sections of diabetic rats showed damage in the hepatic architecture (swelling of sinusoids, vacuolization of cytoplasm, and inflammation of the central vein) which was controlled by the extracts. **Conclusion:** This study agrees with the traditional use of FF bark as an antidiabetic agent.

Key words: Antidiabetic, *Fraxinus floribunda,* lipid profile, liver histopathology, streptozotocin, α-glucosidase

SUMMARY

- The bark of *Fraxinus floribunda* Wallich is traditionally used for the treatment of high blood sugar/diabetes by the herbal practitioners of Sankhu, a village in West Sikkim, India. Since there was not any scientific report available on this plant, this study has been done to evaluate *in vitro* and *in vivo* antidiabetic activity on the bark of this plant. The bark was extracted through four different methods (boiling, soxhletion, pressure boiling (PB) and cold percolation) to observe the influence on bioactivity due to the variation in extraction methods. Alpha-glucosidase inhibiting the activity of the extracts was performed the result of which showed the extracts obtained through PB exhibited the highest antidiabetic activity. It further helped to select the extract for investing antidiabetic activity in animal model. Streptozotocin (STZ)-induced rats were used for the same. The parameters taken for *in vivo* experiments were change in body weight, oral glucose tolerance test, fasting plasma glucose concentration, lipid profile, and liver histopathology. The results showed the control in loss of bodyweight, control in increase of glucose level and improved lipid profile of STZ-induced diabetic rats by 200 and 400 mg/kg extracts. The liver histopathology also revealed improvement in architectural changes noticed in diabetic rats by the same concentration of extracts. This study supports the use of the plant as antidiabetic agent in traditional medicine and also suggests further investigation in purifying the bioactive phytochemical responsible for the antidiabetic activity.



Abbreviations Used: FF: *Fraxinus floribunda*; DM: Diabetes mellitus; T2DM: Type 2 diabetes mellitus; PPHG: Postprandial hyperglycemia; NB: Normal boiling; PB: Pressure boiling; S: Soxhelation; CP: Cold percolation; OGTT: Oral glucose tolerance test; NIDDM: Noninsulin-dependent Diabetes mellitus; STZ: Streptozotocin; TCL: Total cholesterol; TGL: Triglycerides; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; DMRT: Duncan multiple range test.

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INTRODUCTION

Diabetes mellitus (DM) simply known as diabetes is a condition of disordered metabolism characterized by abnormally high level of blood sugar (hyperglycemia) in the body which mainly occurred either due to hereditary causes or sedentary lifestyle.[1] It is also represented by lipidemia and oxidative stress; it affects the patients to long-term complications causing damage in the eyes, kidneys, nerves, skin, and blood vessels.[2] Other than hyperglycemia, it is

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characterized by disturbances in the metabolism of carbohydrate, protein, and fat. Hyperlipidemia is another factor of DM which could develop micro and macrovascular complications of diabetes resulting mostly into morbidity or death.[3] Type 2 diabetes mellitus is the most common diabetes causing > 80% of the total cases of diabetes.[4] Insulin is the hormone released by β-cells of the pancreas, which is responsible for glucose homeostasis.[5] Stimulation of hepatocytes, myocytes, and adipocytes by insulin leads to the uptake of glucose from the circulatory system. Glucose can either be used in glycolysis or stored inside muscle or liver cells in the form of glycogen. If insulin is inappropriately utilized, it results in insulin resistance or the inability of cells to respond to normal circulating insulin levels, thus leading to the incidence of the disease. Increased level of postprandial hyperglycemia (PPHG) is one of the highest risk factors.[6] PPHG is elevated due to the action of α-glucosidase and α-amylase enzymes. Inhibition of these enzymes can manage PPHG in diabetic patients. Inhibition of α-glucosidase enzyme activity can reduce disaccharide hydrolysis, which has useful effects on glycemic index control in diabetic patients.[7] Several α-glucosidase inhibitors have been isolated from medicinal plants for the development of new drugs with increased potency and lower adverse effects than the existing drugs.[8,9]

Recently, diabetes is one of the most prevalent diseases in the world which is rapidly increasing worldwide. According to the WHO, the occurrence of diabetes might increase by 35% in the near future. Currently, over 150 million populations in the world are affected by diabetes, which is likely to increase over 300 million or more by the year 2025. In India, the number of diabetic people will increase from 15 million in 1995 to 57 million in the year 2025, which is considered to be the highest number of diabetics in the world.[10] Despite considerable development in the treatment of diabetes by oral anti-hyperglycemic agents, search for newer drugs is still persisting due to therapeutic limitations of existing synthetic drugs with some seriously harmful side effects after prolonged usage.[11,12] Therefore, proper management of diabetes without any drug-mediated side effects is still being a big challenge.[13] However, the researchers are working to find safer, more efficient, and less expensive remedy for diabetes. Numerous medicinal plants have been reported to have antidiabetic property, and they have been used immensely as antidiabetic and antihyperlipidemic remedies.[14]

Fraxinus floribunda Wallich (FF) belonging to family Oleaceae is a plant usually found in the Eastern Himalayas of India, particularly in Khasi Hills and Sikkim. The bark of this plant is conventionally used for the treatment of diabetes in the villages of West district of Sikkim. Earlier reports have suggested the presence of antioxidant, hepatoprotective, and anti-inflammatory activity on the aqueous extract of the bark of FF.[15] Although it is popularly used as an antidiabetic agent in the traditional system, but there was not a single report on the scientific study of this plant. Thus, this work has been carried out to determine the antidiabetic activity of the bark of FF, which might also validate the use of this plant in traditional system of medicine.

MATERIALS AND METHODS

Plant material

The fresh bark of FF was collected from a village called Sankhu in Dentam constituency of West Sikkim. The herbarium of the plant was submitted in the NBU-Herbarium which was identified by Dr. A.P. Das (former Professor), Plant Taxonomy and Environmental Biology, Department of Botany, University of North Bengal. A voucher specimen, accession no. 9632/Tag no E.S.03 was preserved for future reference.

Animals

Wister albino rats (150–250 g) were used in this study. They were obtained from the Animal House of Columbia Institute of Pharmacy, Raipur. They were housed in large propylene cage and kept at 22°C ± 2°C in 12 h dark light cycle of 12:12 h, relative humidity 55%–65%. The animals were fed with pellet food and water *ad libitum*. The experiments were approved by the Institutional Animal Ethics Committee of Columbia Institute of Pharmacy, Raipur, India (Regd. No. 1321/PO/ReBi/S/10/CPCSEA). All the animals were acclimatized for at least 1 week before the experimental session.

Preparation of plant sample

The fresh bark of FF was sun-dried until the moisture was gone. The dried bark was ground into fine powder and further extracted through four different methods, namely normal boiling, autoclave under pressure boiling (PB), soxhlation in a Soxhlet apparatus (S) and cold percolation below – 4°C (CP). All the extracts were measured and a required volume was made up and the extracts were stored in a refrigerator.

Inhibition of α-glucosidase enzyme

A 0.2 M phosphate saline buffer (pH 6.8) was prepared to dissolve α-glucosidase enzyme in concentration of 0.2 U/ml, 3 mM glutathione (reduced), and 10 mM p-NPG (substrate). To 2.5 ml of buffer, 0.1 ml gluthathione and 0.1 ml enzyme were added and incubated for 15 min at 37°C. After incubation, 0.5 ml of inhibitor (extract) was added except in control followed by 0.25 ml p-NPG. The mixture was incubated for 15 min at room temperature and the reaction was inhibited by the addition of 4 ml sodium carbonate.[16] The absorbance of yellow color of reaction mixture was taken at 405 nm.

The percentage inhibition was calculated as:

$$\text{Percentage inhibition} = \left(\frac{\text{Control} - \text{Test sample}}{\text{Control}}\right) \times 100$$

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) was performed in overnight fasted (18 h) normal rats. Rats divided into three groups (n = 6) were administered drinking water, the second group with aqueous extract of 200 mg/kg, and the third group with 400 mg/kg aqueous extract. Glucose (0.5 mg/kg) was fed 30 min prior to the administration of extracts. Blood was withdrawn from the retro-orbital sinus at 30 and 90 min of extract administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase-peroxidase glucose estimation kit.

Induction of noninsulin dependent diabetes mellitus

Noninsulin-dependent diabetes mellitus (NIDDM) was induced in overnight fasted adult Wistar albino male rats weighing 170–220 g by a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ), 15 min after the i. p. administration of 120 mg/kg of nicotinamide. STZ was dissolved in citrate buffer with pH 4.5 and nicotinamide being dissolved in normal saline. Hyperglycemia in rats was confirmed by the elevated glucose levels in plasma, determined at 72 h and then on day 7 after injection. The starting value of fasting plasma glucose to identify diabetes was taken as >126 mg/dl. Only those rats which were found to have permanent NIDDM were used for the study.

Experimental

The animals were distributed into five different groups with six rats each. The extract was administered for 12 days. Group I served

as normal control rats were given drinking water daily for 12 days; Group II consisted of diabetic control rats administered drinking water daily for 12 days; Group III diabetic rats administered standard drug Glibenclamide (0.50 mg/kg) for 12 days; Group IV diabetic rats administered aqueous extract of 200 mg/kg body weight; and Group V diabetic rats administered aqueous extract of 400 mg/kg bodyweight. The fasting glucose levels were determined on day 0, 7, 14, and 28 of extract administration. During the experimental period, the rats were weighed daily, and the mean change in body weight was calculated.[17,18]

Estimation of biochemical parameters

On day 12, after the animals were sacrificed by cervical dislocation, the biochemical parameters were determined. The tests carried out were total cholesterol (TCL), triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (LDL) by glucose oxidase method using autoanalyzer.[19,20]

Histopathology

All the animals were sacrificed on the 12th day by cervical dislocation, liver were isolated and were subjected to histopathological studies, and microscopical findings were noted.

Statistical analysis

The data are expressed as a mean ± standard error of the mean of six independent experiments. Statistical significance between group was evaluated using one-way analysis of variance followed by Dunnet's test and Duncan multiple range test with $P < 0.05$ value was considered as statistically significant.

RESULTS

Alpha-glucosidase inhibiting assay

The result of α-glucosidase inhibiting activity of aqueous extract of bark of FF is presented in Figure 1. Since the sample was extracted through four different methods to observe the variation in the bioactivity, it was found that the extraction process has a great influence on the bioactivity of plant extracts. The extract obtained through PB has showed the highest activity with the lowest IC_{50} values (10.25 ± 4.56 mg/ml FWT). While the CP extract exhibited the lowest activity.

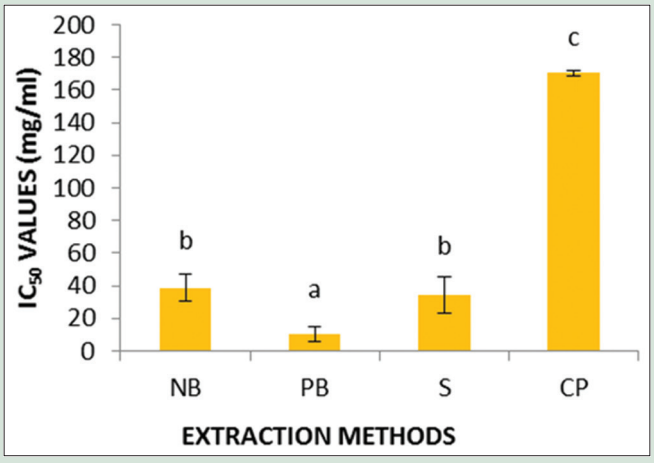

Figure 1: α-Glucosidase inhibiting activity of aqueous extracts of *Fraxinus floribunda*. Values with different letters (a, b, c) are significantly ($P < 0.05$) different from each other by Duncan multiple range test. NB: Normal boiling; PB: Pressure boiling; S: Soxhlet; CP: Cold percolation

Acute toxicity study

The acute toxicity study of aqueous FF bark extract was evaluated as per the CPCSEA guideline no. 420 (fixed dose method). The aqueous extracts were orally fed to the rats at the dose level of 5, 50, 300, and 1000 mg/kg, respectively. The test showed no mortality even at maximum dose of 1000 mg/kg body weight (b.w.). Hence, 200 mg/kg and 400 mg/kg doses were selected for further study.

Oral glucose tolerance test

Results obtained from OGTT is given in Table 1 where it can be observed that there is a significant increase of blood glucose concentration ($P < 0.05$) in the diabetic control group compared to the normal control at the end of 12th day experiment duration. The elevated glucose level was significantly lowered ($P < 0.05$) by the aqueous extracts of bark of FF while comparing with the diabetic control. The significant reduction in plasma glucose level in glucose-loaded rats by 200 and 400 mg/kg extract of FF bark was observed after 30 min, and it came down to normal level after 90 min.

Effect on fasting blood glucose

Table 2 shows administration of STZ-induced hyperglycemia in rats with statistically significant ($P < 0.05$) elevation of blood glucose as compared to normal control to the level >250 mg/dl. However, it was significantly ($P < 0.05$) lowered by the oral administration of glibenclamide (0.05 mg/kg) and aqueous extract of FF bark (200 and 400 mg/kg). As expected, 400 mg/kg of the extract showed the more significant antidiabetic property. The glucose level in STZ-induced diabetic rats lowered to normal after day 14 in case of glibenclamide and 400 mg/kg extract, whereas the 200 mg/kg extract could lower the glucose level to normal after day 28.

Body weight

Effect of standard drug and bark extract of FF on the body weight of diabetes-induced rats is shown in Table 3. In normal control rats, it was observed that bodyweight continuously increased. Diabetic control rats showed a significant decrease ($P < 0.05$) in bodyweight compared to normal control. The diabetic rats administered with glibenclamide and extract of FF bark (200 and 400 mg/kg) showed a significant increase ($P < 0.05$) in bodyweight when compared to diabetic control.

Effect of extracts on lipid profile

Lipid profile of the STZ-induced diabetic rats is presented in Table 4. It can be observed that TGL, TCL, and LDL level were significantly increased ($P < 0.05$) in diabetic rats as while HDL level was significantly decreased as compared to normal control. The bark extracts of FF (200 and 400 mg/kg) significantly decreased the serum TGL, TCL, and LDL and increased the HDL when compared with the diabetic control.

Table 1: Effect of different extracts on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dl)		
	0 min	30 min	90 min
Normal control	73.15±3.24	76.42±2.73	74.83±4.51
Glucose control	76.61±6.17	221.28±5.38#	155.19±3.64#
Glucose + glibenclamide (0.5 mg/kg)	78.34±4.42	90.71±3.25*	75.52±5.18*
Extract (200 mg/kg)	75.58±3.71	128.21±4.86*	96.17±4.43*
Extract (400 mg/kg)	74.83±5.36	108.47±3.18*	78.63±3.29*

Values are expressed as mean±SEM (number of animals, n=6); Significantly different at #P<0.05 when compared with normal control group; *P<0.05 when compared with diabetic control group. SEM: Standard error of the mean

Table 2: Effect of different extracts on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal control	78.62±2.15	75.39±5.12	80.17±3.41	74.69±5.28
Diabetic control (STZ)	149.29±3.62#	208.34±2.57#	249.48±5.62#	287.11±2.71#
Diabetic + standard glibenclamide (0.50 mg/kg)	134.43±2.83	112.65±4.32*	90.29±2.39*	75.38±4.68*
Diabetic + extract (200 mg/kg)	129.51±4.34	132.74±3.51*	113.68±4.73*	92.76±2.15*
Diabetic + extract (400 mg/kg)	132.24±3.05	118.43±4.26*	97.36±2.86*	78.42±3.52*

Values are expressed as mean±SEM (number of animals, *n*=6); Significantly different at #*P*<0.05 when compared with normal control group; **P*<0.05 when compared with diabetic control group. SEM: Standard error of the mean

Table 3: Effect of extracts on changes in bodyweight in rats

Group	Change in body weight (g)		
	Before induction	After induction	After treatment
Normal control	182.32±2.14	171.49±3.28	177.83±2.63
Diabetic control (STZ)	185.21±1.98	139.67±2.68#	112.38±3.86#
Diabetic + standard glibenclamide (0.50 mg/kg)	168.19±3.05	132.72±3.14	175.31±4.56*
Diabetic + extract (200 mg/kg)	176.15±3.53	142.59±2.43	151.84±2.49*
Diabetic + extract (400 mg/kg)	173.64±2.79	146.12±1.54	169.76±2.18*

Values are expressed as mean±SEM (number of animals, *n*=6); Significantly different at #*P*<0.05 when compared with normal control group; **P*<0.05 when compared with diabetic control group. SEM: Standard error of the mean; STZ: Streptozotocin

Table 4: Determination of biochemical parameters after treatment with different extracts

Group	Lipid profile (mg/dl)			
	TGL	TCL	HDL	LDL
Normal control	79.24±3.28	76.65±4.36	72.84±2.68	56.28±4.53
Diabetic control (STZ)	186.37±3.69#	198.41±2.72#	31.56±4.25#	165.69±5.74#
Diabetic + standard glibenclamide (0.50 mg/kg)	78.53±4.17*	72.84±4.69*	76.29±6.43*	63.82±2.28*
Diabetic + extract (200 mg/kg)	96.52±2.69*	101.62±2.81*	51.35±3.79*	85.26±4.15*
Diabetic + extract (400 mg/kg)	83.41±2.47*	79.95±5.24*	70.16±4.41*	58.64±3.26*

Values are expressed as mean±SEM (number of animals, *n*=6); Significantly different at #*P*<0.05 when compared with normal control group; **P*<0.05 when compared with diabetic control group. SEM: Standard error of the mean; TGL: Triglycerides; TCL: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; STZ: Streptozotocin

As expected, standard glibenclamide administered rats significantly prevented the increase of TGL, TCL, and LDL and decrease of HDL compared to diabetic control. The extracts were able to restore the lipid profile of diabetic rats to almost normal level.

Histopathology

The effect of bark extracts of FF on the histological architecture of the liver is given in Figure 2. Liver sections of the normal group showed normal hepatic structure [Figure 2a]. Normal hepatic cells were observed distinctively forming a network around central veins with peripheral portal areas in the surrounding. However, liver sections of diabetes-induced rats showed hepatocellular injury with the loss of normal architecture of the liver as compared to the normal group [Figure 2b]. Inflammation and vacuolization of cytoplasm were observed. There was dilation in the central vein along with dilation and congestion of blood sinusoids. There was also an enlargement of the space between the hepatocytes and sinusoidal dilation. This hepatic injury was observed to be almost recovered to normal by the extracts (200 and 400 mg/kg) of FF bark [Figure 2d and e]. The sinusoids were restored with the reduction of enlargement, inflammation of central veins were also reduced. Administration of glibenclamide on diabetic rats was able to repair the hepatic injury to almost like normal control [Figure 2c].

DISCUSSION

We have already discussed that aqueous decoction of the bark of FF is popularly used in traditional medicine for treating diabetic patients in some villages of Sikkim. Since there is no scientific validation to its usefulness, this work was designed to ascertain the scientific base for this. In the traditional system, the simple boiling method is used to extract the therapeutic activity of the plant, but this work has focused on the other methods of extraction to observe the influence of various extraction methods on the bioactivity of the plant. Phytochemicals present in plants such as flavonoids, glycosides, terpenoids, alkaloids, and carotenoids are often implicated as having antidiabetic effect.[21] However, extraction method plays a crucial role in the productivity of these bioactive phytochemicals since such health promoters must be obtained sufficiently with minimum damage from the raw material.[22]

The samples extracted through four different methods were subjected to α-glucosidase inhibiting assay. Alpha-glucosidase is an enzyme that plays a vital role in modulating PPHG by breaking down α-1,4-glucosidic linkages of disaccharides. The effects of α-glucosidase inhibitors and their use on delaying the generation of blood glucose after food uptake has been established by various authors.[23] A distinct variation in antidiabetic activity by the extracts was observed. The bark extract of FF obtained through PB showed the highest antidiabetic activity with IC_{50} value 10.25 mg/ml FWT. The range of IC_{50} value increased up to 170.27 mg/ml FWT exhibited by CP with lowest antidiabetic activity. Similar results were reported previously where boiling under high pressure has extracted the highest antioxidant activity in case of FF.[24] According to mass transfer phenomena and phase theories, pressure increases the permeability and solubility of plant tissue, and there is a diffusivity

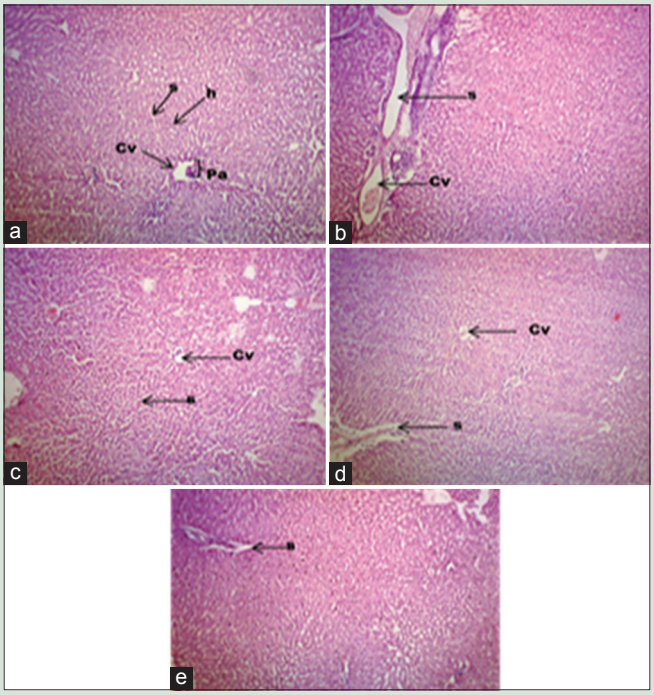

Figure 2: Liver histopathology of streptozotocin induced rats and effects of bark of *Fraxinus floribunda* on it. (a) Normal control, (b) Diabetic control, (c) Diabetic + glibenclamide, (d) Diabetic + 200 mg/kg extract, (e) 400 mg/kg extract. s: Sinusoids; h: Hepatocytes; Cv: Central vein; Pa: Portal area

of cell components which results in the movement of cellular components out of the cell.[25]

The extract obtained from PB was selected for further investigation of antidiabetic activity in animal model. There was an improvement in glucose tolerance test with the reduction of plasma glucose level which indicates the insulin-mimetic activity or improvement of glucose utilization mechanism by the extract.[26] The antidiabetic activity of FF extract might be credited to the decrease in damage of pancreatic β-cell, thus improving the production of insulin from the β-cell of the pancreas. Numerous plants have been previously reported to have antihyperglycemic activity by insulin stimulating effect.[27,28] The mechanisms of actions for plants with antihyperglycemic activity mainly include the increase of insulin secretion, control in glucose absorption by the intestine, more glucose absorption by muscle and fat tissues, and control in the production of glucose from liver cells.[29]

One of the characteristic features of diabetes is the inability of glucose uptake by the muscle cells because of low insulin production which consequently causes muscle wasting and decrease in bodyweight. Induction of STZ in rats will destroy the pancreatic β-cells due to low levels of insulin.[30] Thus, there was a decrease in bodyweight in diabetic control rats as compared to normal control ones, which indicate the excessive breakdown of tissue proteins causing the loss of body weight in diabetes.[31-34] It was clearly observed that the administration of FF extract improved the loss of bodyweight which indicated the control on wastage of muscle in diabetes. The FF extract has probably stimulated the pancreatic beta cells leading to the production of insulin.

Diabetes affects lipid profile, and the most common lipid abnormalities are high TGL and high TCL. In this study, there was an increase in TCL and decrease in HDL in diabetic control rats. Deficiency of insulin may cause the failure to activate lipoprotein lipase resulting into hypertriglyceridemia.[35] However, the bark extract of FF was able to control the lipid levels in diabetic rats. In diabetes, LDL brings cholesterol to the peripheral tissues to be deposited while HDL carries cholesterol to liver from peripheral tissues and helps its excretion. LDL is responsible for the deposition of fats in arteries. In this study, we have observed a significant decrease in TCL, TGL, and LDL, whereas HDL level was significantly increased.

The liver is important and helps the body in controlling blood glucose with glycogenesis and glycogenolysis. The liver sections of STZ-induced rats revealed various architectural changes in the liver with inflammation of sinusoids, changes in central veins, and portal area with vacuolization of cytoplasm. Similar findings were reported earlier by many researchers with histopathological changes in the liver.[36-38]

Unlike diabetic control, the liver sections of diabetic rats treated with glibenclamide and FF bark extracts showed less histopathological changes and improved liver architecture. It indicates the protective effect of the extracts to control hepatic injury during diabetes.

CONCLUSION

From the results of this study, it can be concluded that the aqueous extract of bark of FF possess antidiabetic activity along with antihyperlipidemic property against STZ-induced hyperglycemia. We also suggest extraction of the FF bark with a method involving pressure to acquire better results. Our study supports the traditional use of this plant for the treatment of diabetes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Raman BV, Krishna AN, Rao BN, Saradhi MP, Rao MV. Plants with antidiabetic activities and their medicinal values. Int Res J Pharm 2012;3:11-5.
2. Elosta A, Ghous T, Ahmed N. Natural products as anti-glycation agents: Possible therapeutic potential for diabetic complications. Curr Diabetes Rev 2012;8:92-108.
3. Pavana P, Sethupathy S, Manoharan S. Antihyperglycemic and anti-lipid peroxidative effect of *Tephrosis purpura* seed extract in streptozotocin induced diabetic rats. Indian J Clin Biochem 2007;22:77-83.
4. Mlinar B, Marc J, Janez A, Pfeifer M. Molecular mechanisms of insulin resistance and associated diseases. Clin Chim Acta 2007;375:20-35.
5. Gupta PD, De A. Diabetes mellitus and its herbal treatment. Int J Res Pharm Biomed Sci 2012;3:706-21.
6. He ZX, Zhou ZW, Yang Y, Yang T, Pan SY, Qiu JX, *et al.* Overview of clinically approved oral antidiabetic agents for the treatment of type 2 diabetes mellitus. Clin Exp Pharmacol Physiol 2015;42:125-38.
7. Ahamad J, Naquvi KJ, Mir SR, Ali M, Shuaib M. Review on role of natural α-glucosidase inhibitors for management of diabetes mellitus. Int J Biomed Res 2011;2:374-80.

8. Onal S, Timur S, Okutucu B, Zihnioğlu F. Inhibition of alpha-glucosidase by aqueous extracts of some potent antidiabetic medicinal herbs. Prep Biochem Biotechnol 2005;35:29-36.
9. Li Y, Wen S, Kota BP, Peng G, Li GQ, Yamahara J, *et al. Punica granatum* flower extract, a potent alpha-glucosidase inhibitor, improves postprandial hyperglycemia in Zucker diabetic fatty rats. J Ethnopharmacol 2005;99:239-44.
10. Vijayaraj R, Vidhya R. Biological activity of *Achyranthes aspera* linn: A review. Asian J Biochem Pharm Res 2016;1:86-93.
11. Spiller HA, Sawyer TS. Toxicology of oral antidiabetic medications. Am J Health Syst Pharm 2006;63:929-38.
12. Eurich DT, McAlister FA, Blackburn DF, Majumdar SR, Tsuyuki RT, Varney J, *et al*. Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: Systematic review. BMJ 2007;335:497.
13. Saxena A, Vikram NK. Role of selected Indian plants in management of type 2 diabetes: A review. J Altern Complement Med 2004;10:369-78.
14. Vijayaraj R, Kumaran NS. Evaluation of *in vivo* antidiabetic and antioxidant activity of *Achyranthes aspera* linn. seeds by streptozotocin induced diabetic rats. Int J Green Pharm 2018;12:29-37.
15. Subba A, Dutta B, Sahu RK, Mandal P. Antioxidant, anti-inflammatory and hepatoprotective activity of *Fraxinus floribunda* bark and the influence of extraction process on their bioactivity. J Pharm Res 2017;11:983-90.
16. Oki T, Matsui T, Osajima Y. Inhibitory effect of α-glucosidase inhibitors varies according to its origin. J Agric Food Chem 1999;47:550-3.
17. Singh J, Sahu RK, Prasad DN, Jangde R, Gupta R. Evaluation of antidiabetic potential of *Ougeinia oojeinensis* leaves in streptozotocin-induced-diabetic rats. Pharmacolonline 2011;2:1046-52.
18. Sharma U, Sahu R, Roy A, Golwala D. *In vivo* antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. J Young Pharm 2010;2:255-60.
19. Nyarko AK, Sittie AA, Addy ME. The basis for the antihyperglycaemic activity of *Indigo feraarrecta* in the rat. Phytother Res 1993;7:1-4.
20. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972;97:142-5.
21. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants. Acta Pol Pharm 2010;67:113-8.
22. Kocak E, Pazır F. Effect of extraction methods on bioactive compounds of plant origin. Turk J Agric Food Sci Technol 2018;6:663-75.
23. Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of inhibition of alpha-glucosidase and pancreatic amylase. Food Chem 2009;115:1268-73.
24. Subba A, Mandal P. Antioxidant potential of *Fraxinus floribunda* bark extracted through various aqueous processing. Free Rad Antiox 2015;5:6-12.
25. Banerjee S, Chatterjee J. Efficient extraction strategies of tea (*Camellia sinensis*) biomolecules. J Food Sci Technol 2015;52:3158-68.
26. Singh MP, Shukla ST, Pateriya A. An investigation on antidiabetic and antioxidant activity of extract of leaves of *Amaranthus polygamus* in streptozotocin induced diabetic rats. Indian Drugs 2018;55:49-55.
27. Jia Q, Liu X, Wu X, Wang R, Hu X, Li Y, *et al*. Hypoglycemic activity of a polyphenolic oligomer-rich extract of *Cinnamomum parthenoxylon* bark in normal and streptozotocin-induced diabetic rats. Phytomedicine 2009;16:744-50.
28. Gireesh G, Thomas SK, Joseph B, Paulose CS. Antihyperglycemic and insulin secretory activity of *Costus pictus* leaf extract in streptozotocin induced diabetic rats and in *in vitro* pancreatic islet culture. J Ethnopharmacol 2009;123:470-4.
29. Girija K, Lakshman K, Udaya C, Sabhya SG, Divya T. Anti-diabetic and anti-cholesterolemic activity of methanol extracts of three species of *Amaranthus*. Asian Pac J Trop Biomed 2011;1:133-8.
30. Baruah D, Bora M, Bora D, Bharali BK, Hazra J. Evaluation of hypoglycemic activity of *Annona reticulata* L. stem bark extracts against streptozotocin induced diabetic rats. Int J Res Ayurveda Pharm 2017;8:132-6.
31. Sajeesh T, Arunachalam K, Parimelazhagan T. Antioxidant and antipyretic studies on *Pothos scandens* L. Asian Pac J Trop Med 2011;4:889-99.
32. Kumar D, Kumar S, Kohli S, Arya R, Gupta J. Antidiabetic activity of methanolic bark extract of *Albizia odoratissima* Benth. in alloxan induced diabetic albino mice. Asian Pac J Trop Med 2011;4:900-3.
33. Poongothai K, Ponmurugan P, Ahmed KS, Kumar BS, Sheriff SA. Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown & *in vitro* raised) extracts on alloxan induced diabetic rats. Asian Pac J Trop Med 2011;4:778-85.
34. Mishra SB, Verma A, Mukerjee A, Vijayakumar M. Anti-hyperglycemic activity of leaves extract of *Hyptis suaveolens* L. poit in streptozotocin induced diabetic rats. Asian Pac J Trop Med 2011;4:689-93.
35. Appalaraju G, Reddy CS, Reddy CV, Rajani G, Nithin C, Raju AB. Anti diabetic activity of polyherbal extract on streptozotocin induced diabetes in Wistar rats. Ann Biol Res 2014;5:39-46.
36. Aboonabi A, Rahmat A, Othman F. Effect of pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic induced rats. J Cytol Histol 2014;6:1-5.
37. Hassan NF, Soliman GM, Okasha EF, Shalaby AM. Histological, immunohistochemical, and biochemical study of experimentally induced fatty liver in adult male albino rat and the possible protective role of pomegranate. J Microsc Ultrastruct 2018;6:44-55.
38. Ania IM, Abireda AN, Mustafab BE, Wahaba EN, Azzubaidic MS. Effect of flaxseed extract on the liver histological structure in streptozotocin induced diabetic rats. Int Med J Malaysia 2017;6:91-8.