Chapter 7

To study antidiabetic properties of phytochemical extract of bhimkol blossom

7.1 Introduction

Diabetic Mellitus (DM) is the most prevalent severe metabolic disorder that has been rising worldwide in the great hike [19]. According to WHO report [37], about 422 million populations are suffering from diabetes and 1.5 million deaths are directly propagated to diabetes each year worldwide. DM is a condition where a system does not produce sufficient insulin or improperly responds to insulin [25] leading to damage to kidneys, eyes, heart, and nerves over a long period. Glucose metabolism disorders are the major factor projecting diabetes [5]. Some common types of diabetes *viz*., type 1, 2, prediabetes, and gestational diabetes [13]. Type 1 diabetes (DM-1) is a condition that affects the way the body regulates blood sugar, whereas type II diabetes (DM-2) is a condition where the pancreas produces little or no insulin mostly occurring in adults. Prediabetes is a condition where blood sugar is high but lesser than DM-2 condition, and gestational diabetes is a condition where pregnant women suffer high blood sugar [18]. Aging and obesity was the main factor for increasing diabetes [22]. It is also characterized by hypercholesterolemia, hyperlipidemia, hyperinsulinemia, and hyperaminoacidemia [33]. Moreover, damaged β-cells (pancreatic cells) lead to insufficient production of insulin by the pancreas. Sometimes, due to dysfunction of insulin receptor, blood glucose cannot be regulate in the proper system and then encourages increasing level of glucose in the bloodstream. Diabetic patients are mostly diagnosed by providing insulin and other drugs (viz., α-glucosidase inhibitors, biguanides, thiozolidinediones, meglitinides, glinides, etc.) which lowers the glucose accumulation in the blood [15, 33]. An individual with a fasting blood glucose level (BGL) generally ranging above 100-125 mg/dL is stated as prediabetes and 126 mg/dL and above indicates diabetes [38]. Herbal medicines are the growing interest; especially plant-based medicinal extracts due to the side effects (such as hypoglycemia) of oral therapeutic drugs used for the diagnosis of diabetes [28]. Herbal medicinal extracts contain numerous bioactive compounds such as polyphenols, terpenoids, and other antioxidants which are reported to control diabetes. Phytochemicals and many bioactive compounds may act as an inhibitor of enzymes causing increment of blood glucose (viz., α-amylase, α-glucosidase, and DPP-IV (dipeptidyl pepdidase-IV) enzymes) [12]. Some compounds exhibit antioxidant and anti-inflammatory properties (flavonoids, terpenoids, some vitamins, and some unsaturated fatty acids) that help to treat damaged β-cells and

dysfunctional insulin receptors [8, 17]. Quercetin is a key member of the flavonoid group which possesses a great antidiabetic property along with various medicinal properties [34]. Quercetin is also reported to manage DM-1 by protecting against liver injury through the inhibition of CYP2E1 (a powerful cellular pro-oxidant that causes cell damage) [24] and DM-2 [11] diabetes effectively. Therefore, phytochemical-rich plant extracts have been reported to treat diabetic induced rats [12]. Banana blossoms are also a great source of numerous phytochemicals [29, 10]. *Musa balbisiana* (bhimkol) blossom (BB) is rich in nutrients and contains a significant amount of phytochemicals with major quercetin content [29]. This particular variety is believed to be more special compared to other varieties (*viz*., *Musa acuminate*, *Musa paradisiaca*, *Musa sapientum*, etc.) and is most preferred for consumption because of its lesser astringency and balanced flavor. Historically, BB is consumed mainly to treat hypertension and hyperglycemia in some Asian countries; moreover, it is considered an underutilized agricultural byproduct and discarded as waste by the maximum population [27]. There is almost negligible report on antidiabetic property of BB. Some other varieties of banana blossoms viz., *Musa* sp. var. ellaki bale [10], *Musa sapientum* [9], and *Musa acuminate* colla [23], are also reported to treat diabetes.

This study includes detailed *in vitro* and *in vivo* study of antidiabetic properties of quercetin rich bhimkol blossom extract. A detailed *in vitro* study was done on the inhibitory activity of BB extracts on diabetic-enhancing enzymes. Quercetin-rich BB-dosed Wister rats were observed for effect on their blood glucose and other effects. The study elaborates effect of quercetin-rich BB extract on DM-1 and DM-2.

7.2 Materials and methods

7.2.1 Raw materials and chemical reagents

Inflorescence of BB was collected from the Tezpur University Campus, Assam (26.7003ºN, 92.8308ºE), India. Immediately after collection BB was washed under running tap water and then chopped. Chopped parts were dried in a hot air oven (Advantage lab, AL01-05-100, Belgium) at 45^{o}C for 24 h and ground to powder, then sieved (300 µm) and stored in air-tight containers at 4^{o}C for further analysis as detailed in Chapter 2.

All the reagents and chemicals used in the study were of analytical grades. All the chemicals and reagents were purchased from Sigma Aldrich (USA) and Hi-Media Laboratories Pvt. Ltd. (India).

7.2.2 Preparation of BB extract

Powder of BB was mixed in an extraction solvent of 70% ethanol at a 2:30 ratio in a 100 ml beaker. As previously work done in Chapter 2 [4, 29], the sample BB powder and solvent ratio (2:30) were ultrasonicated (Q Sonica, Q700, USA) with 220 Å probe (12.7 mm diameter) at a frequency of 20 kHz, temperature (60^oC), extraction amplitude (35%), extraction time (20 min), and at 75% duty cycle (15 s pulse on and 5 s pulse off) by using extraction solvent (70% ethanol). During the extraction process, the probe was immersed in 1.5 times the diameter of the tip and without touching the bottom of the solution in a 100 ml beaker for better extraction.

After the complete extraction process, the supernatant of each sample was filtered using Whatman No.1 filter paper, and the filtrate was allowed to evaporate using a hot air oven at 40^oC. Evaporated BB extract (BBE) was stored at 4^oC for further analysis.

7.2.3 *In vitro* enzyme inhibition activities

7.2.3.1 α-Amylase inhibition activity

α-Amylase inhibition activity was performed by following [1] with slight modification. The substrate was prepared by dissolving 200 mg of starch in 25 ml sodium hydroxide (0.4 M), heated at 100^oC for 5 min, and then pH was adjusted to 7 and finally adjusted up to volume 100 ml with distilled water. In 2 ml of the substrate, 0.2 to 1 mg/ml of acarbose or BBE (1 mg/ml) or blank was added along with a α-amylase solution (3 U/ml). The solution was allowed to mix properly and then incubated (BOD incubator, Optics Tech, 5634, India) at 37^oC for 15 min. To stop the enzyme activity, 80 µl of 0.1 M hydrochloric acid was added along with 100 µl of 2.5 mM iodine reagent. Furthermore, the solution was incubated in the interval (15 and 30 min) at 37^oC and absorbance was noted in a microplate reader (GloMax, Promega, India) at 630 nm. The inhibition (%) of α-amylase is expressed as Equation 1.

$$\%I = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the mixture without the sample and A_{sample} is the absorbance of the mixture with the sample.

7.2.3.2 α-Glucosidase inhibition activity

α-Glucosidase inhibition activity was assayed [26] in a 96-well microplate. A 45 µl of BBE (20 to 500 µg/ml) was added in 1.2 ml of 50 mM of sodium acetate buffer (pH 6.0) and mixed well. Then the mixture was added to a reaction solution of 35 µl of p-nitrophenyl-α-D-glucopyranoside (4 mM) and 35 µl of α-glucosidase (50 mU/ml). The reaction solution was incubated for 30 min at 37^{o}C. Then, the reaction was concluded by the addition of 52 µl of sodium carbonate (0.1 M). The absorbance of each sample was noted at 405 nm in a microplate reader and then percentage inhibition of α-glucosidase was calculated by the given Equation 2.

$$\%I = \frac{(C - S)}{C} \times 100 \quad (2)$$

Where S is the absorbance of the sample ($S = S_1 - S_o$), S_1 is the absorbance of the sample with enzyme addition, S_o is the absorbance of the sample without enzyme addition, and C is the absorbance of the control, without the sample.

7.2.3.3 DPP-IV inhibition activity

For the study of DPP-IV inhibition activity, 40 µl of BBE (20 to 200 µl/ml) was put in 96-well microplates and 20 µl of DPP-IV enzyme (0.05 U/ml) was added. The microplate containing solutions was incubated for 10 min at 37^{o}C. After that, 100 µl of GPPN substrate (0.2 nM in Tris HCl) was added and again incubated at 37^{o}C for 30 min. Finally, each absorbance was noted at 405 nm in a microplate reader. The percentage inhibition of the DPP-IV enzyme was calculated as given in Equation 3 [2].

$$\%I = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (3)$$

Where $A_{control}$ is the absorbance of the DPP-IV solution without the sample and A_{sample} is the absorbance of the DPP-IV solution with the sample.

7.2.4 Glucose uptake by L6 Cells

The glucose uptake study was performed on L6 cells (myoblast cells from skeletal muscle) [14] by using a glucose uptake assay kit (Glucose Uptake Cell-Based Assay Kit 600470 1EA, Cayman, USA). For the fluorescence microscopy study, black and clear bottom microplates were used. Firstly, L6 cells were seeded in 96-well plates in 100 µl culture medium (DMEM and 10 % foetal bovine serum). At the confluence stage, the medium was changed and the cells were incubated for 48 h. After starvation, the cells were treated with control/insulin/insulin with palmitate/BBE. The concentration of BBE for treatment was varied from 20 to 500 µg/ml (in distilled water). Ligands bonded to the receptors on the surface of the cells. After that, ten min prior to the end of the treatment, treated cells were administrated with 100 µg/ml of 2_NBDG (D-Glucose, 2-deoxy-2-((7-nitro-2,1,3-benzoxadiazol-4-yl)amino)-) for 20 min. At the end of the treatment, plates were centrifuged (400 g) at room temperature for 5 min. After the aspiration of supernatant, 200 µl cell-based assay buffer was poured into each well. Again plates were centrifuged in 100 µl of cell-based assay buffer as before. Then, the uptakes of glucose by the treated cells were measured (in mean fluorescence intensity-MFI) by the fluorescence reading at excitation/emission (485/535 nm).

7.2.5 Selection of Wister rats

Wister rats were selected and made ready for the *in vivo* study according to the Organization of Economic Co-Operation and Development (OECD-423) guideline [32]. *In vivo* study was conducted in compliance with an ethical committee, CPCSEA in Defence Research Laboratory, Tezpur, India with Registration No.: 1227/GO/Rbi/S/08/CPCSEA and Protocol No.: 17/IAEC/DRL/25/2/2022. Random and equal ratio of male and female Wister rats (*Rattus norvegicus*) weighing 130–190 g, 6-8 weeks old were selected for the study (n=6) and kept for acclimatization for 7 days in an animal house separately. The animal house was maintained at 22-24^{o}C, 12 h daily at light/dark cycle at RH (50±3^{o}). Rats were given a free standard pellet diet and water at libitum.

7.2.6 *In vivo* antidiabetic study

7.2.6.1 Induction of diabetes in Wister rats

After the acclimatization of Wister rats (n=6) as described above 7.2.5, body weights (BW) and blood glucose levels (BGL) were noted before the initiation of the experiment. Rats were kept for fasting overnight with free access to drinking water. Diabetes was induced by injection of fresh streptozotocin, STZ (55 mg/Kg BW in 0.1 M citrate buffer, pH 4.5) in rats at 20 ml/Kg BW [20]. After the administration of STZ, glucose water (6%) was served to each rat and after that, they were free to access food and water. The BGL was tested every day to ensure a static diabetic condition (BGL > 250 mg/dL). Almost after 72 h, a static diabetic condition was obtained, and rats were carried out for the antidiabetic study.

7.2.6.2 Oral administration of BBE

As detailed in previous Chapter 6, acute toxicity of the BBE dosed rats at (500 mg/Kg BW) was found to be non-toxic [31], hence for the *in vivo* antidiabetic study of BBE, diabetic rats were dosed with BBE (500 mg/Kg BW) at rate of 20 ml/Kg BW. A continuous monitoring of BGL and BW was done. Experimental rats were divided in four equal groups:

Group A: Untreated normal rats (control rats)

Group B: Untreated diabetic rats

Group C: Diabetic rats treated with BBE (500 mg/kg BW)

Group D: Diabetic rats treated with insulin (5 unit/kg BW)

At the end of the stipulated period (4 weeks), blood was collected from the tail vein of each rat.

7.2.6.3 Investigation of the effect of BBE dose in Wister rats

The effects of BBE over BW and BGL of diabetic Wister rats were investigated thoroughly for 4 weeks. The antidiabetic effect of BBE was analyzed by glucose tolerance test and the insulin tolerance test was investigated. The glucose tolerance test

was performed by administration of glucose (2.5 g/kg BW) orally to each experimental group of rats after 4th week of the experiment after 16 h fasting and finally, measured for blood glucose level at intervals (0 to 120 min) [39]. Similarly, the insulin tolerance test was performed [36] at a dose of (0.5 U/kg BW) in each experimental group of rats after 4 h of fasting and finally, measured for blood glucose level at intervals (0 to 120 min).

7.2.7 Statistical analysis

All the experiments other than *in vivo* study were performed in triplicates (n=3) and presented as mean ± SD. Data obtained were statistically analyzed by one-way ANOVA in SPSS software (v11) and significant difference ($p<0.05$) in data were investigated by Tukey's method.

7.3 Results and discussions

7.3.1 *In vitro* enzyme inhibition activities

α-Amylase inhibition property of BBE (0.5 mg/ml) was observed at 7.69±0.06 (I% at 30 min) as shown in Fig. 7.1a. The coefficient of regression of acarbose was obtained at 0.90 (R2) and regression equation {y = (-0.024) x + 0.164} showed 9.71±0.08 (I% at 30 min). A positive inhibition property of BBE against α-Glucosidase was also obtained (Fig. 7.1b). Moreover, the inhibition of α-glucosidase increased from 70±0.43 to 82±0.13 (I%) at a range of 20 to 50 µg/ml, respectively. The inhibition activity decreased after 50 µg/ml of BBE. The inhibition activity dropped to 21±0.38 (I%) at 500 µg/ml of BBE. Generally, the inhibition activity can be seen at a lesser concentration of sample extract rather than a high concentration of sample extract. Inhibition property indicates against both α-amylase and α-glucosidase indicates that BBE has a positive role to slow down glucose absorption in the small intestine during the digestion of starch up to a certain limit. Slowing down the digestion of glucose release helps in minimal absorption of glucose hence slowing down glucose accumulation in the blood. Slowing down the glucose accumulation in the blood indicates the maintenance of diabetes by maintaining hyperglycemia [1].

A significant inhibition activity of BBE against the DPP-IV enzyme was observed as shown in Fig. 7.1c. The inhibition activity of BBE against the DPP-IV enzyme increased from 25 to 200 µg/ml of BBE at 30.47±0.14 to 71.32±0.15 (I%). The

inhibition of the DDP-IV enzyme indicates the inhibition of an enzyme that damages beta cells present in the pancreas. Mostly, in DM-1, the immune system destroys mistakenly own beta cells that usually help in producing insulin. In that case, inhibitors of beta cells damaging enzymes help to recover the beta cells in the pancreas and regulate insulin production leading to lower glucose accumulation in the bloodstream. Hence, inhibition of the DPP-IV enzyme helps in DM caused by beta cell damage [12]. The presence of phytochemicals is the main responsible for the inhibition of α-amylase, α-glucosidase, and DPP-IV enzymes as similarly stated by Kalita et al. [21].

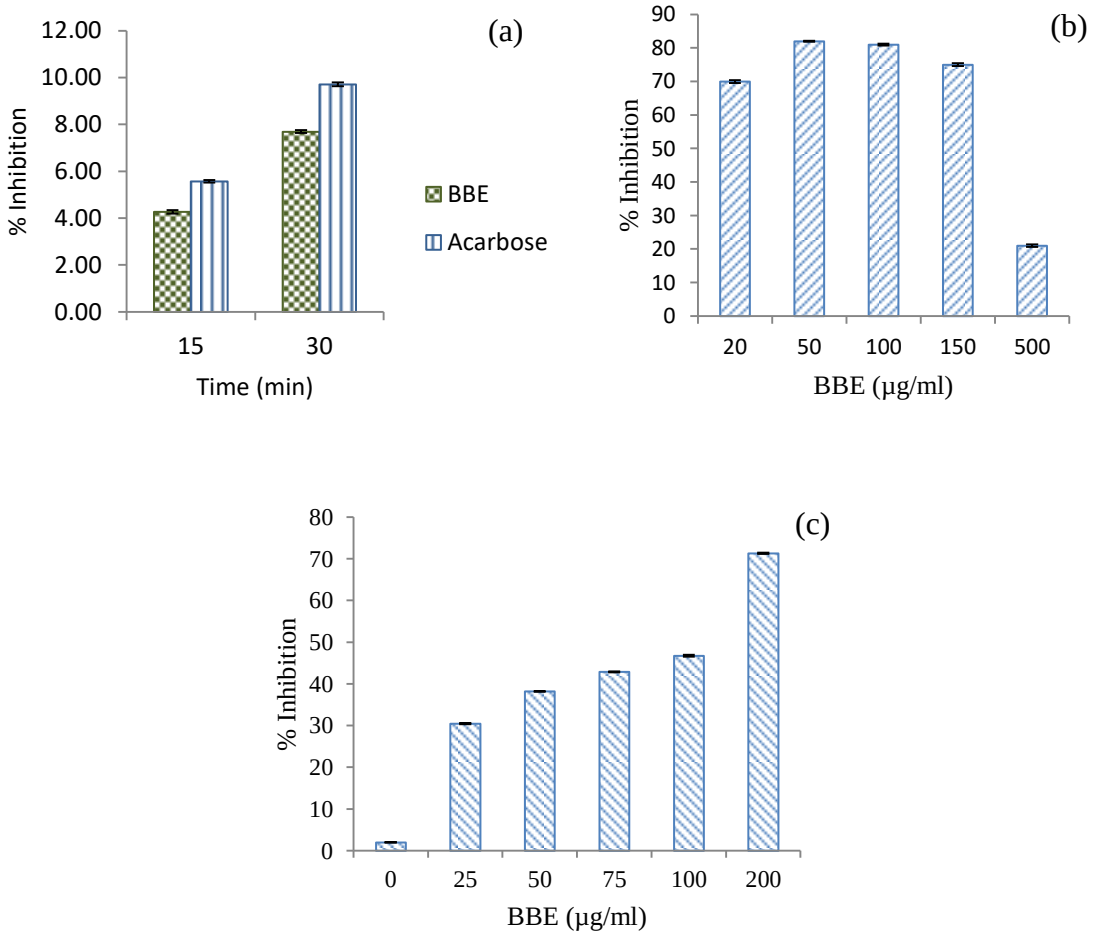

Fig. 7.1 Inhibition activity of BBE against some diabetes enhancing enzymes; (a) α-amylase, (b) α-glucosidase, and (c) DPP-IV enzyme

Various phytochemicals, mainly polyphenols (*viz*., quercetin, catechol, anthocyanin, etc.) and other antioxidants (viz., vitamin E, vitamin C, saponin, etc.) are responsible for the inhibition of α-amylase, α-glucosidase and DPP-IV enzymes [7].

Phytochemicals inhibit such enzymes by mostly direct blockage of active centres of enzyme local sites. These enzyme inhibitors indirectly help in blunting the postprandial blood glucose rise mostly in DM-1 and DM-2 [3].

7.3.2 Glucose uptake by L6 Cells

Uptake of 2_NBDG by insulin treated L6 cell was significantly higher (6.20±0.76 MFI) than BBE treated cell. Uptakes of 2_NBDG by BBE treated L6 cells increased by increasing the concentration of BBE from 20 to 100 µg/ml, whereas a drop in 2_NBDG uptake was observed at 500 µg/ml of BBE treated cells (Fig. 7.2). The highest uptake of 2_NBDG (5.58±0.01 MFI) was seen at 100 µg/ml of BBE treatment. Generally, sample extracts 2 to 50 µg/ml show higher compared to the maximized concentrations of sample extracts, where decreased uptake is due to the exceeding saturation level of glucose. On the other hand, cells treated with insulin + palmitate were in a condition where cells could not uptake 2_NBDG and were in a serious state of DM-2. Significant glucose uptake by BBE treated L6 cells directly indicates the strong ability to treat the damaged L6 cells and help cells to regulate glucose uptake desirably as stated by a similar study reported by Gopalan et al. [16]. This suggests that BBE has a great ability to treat DM-2.

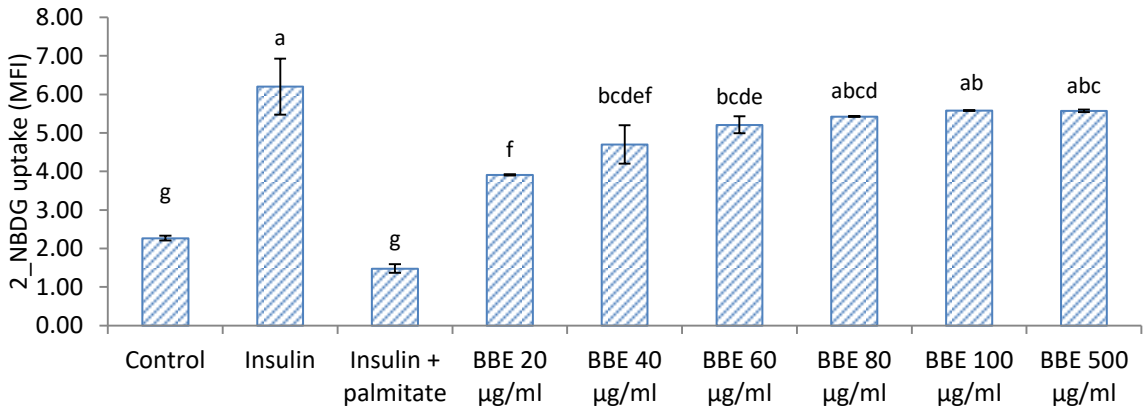

Fig. 7.2 Glucose uptake intensity of control, insulin, insulin + palmitate and BBE on L6 cells. Error bar with different letters are significant different values (p<0.05)

7.3.3 *In vivo* antidiabetic study

7.3.3.1 Effect of BBE on blood glucose of diabetic rats

A positive effect was seen in the blood group of each group of experimental rats (Fig. 7.3) after the oral dose of BBE (500 µg/ml) in 4 weeks as a similar report stated by Vilhena et al. [35]. There was no significant change in blood glucose in control group (group A) without treatment. There was a lesser significant decrease in blood glucose in group B (diabetic group) contrary to insulin and BBE treated diabetic rats (Group C and D, respectively). The highest rate of change in blood glucose (Fig. 7.4) was seen in insulin treated diabetic rats (group D) compared to BBE treated rats (group C). As insulin was the standard protocol to diagnose DM, hence effects of BBE on diabetic rats were studied comparatively. In BBE treated diabetic rats (group C), blood glucose dropped from 493.83±53.18 to 278±7.63 mg/dL at a rate of change 1.77±0.19 and on the other hand, insulin treated diabetic rats (group D) from 451.5±65.12 to 176.33±3.94 at rate 2.56±0.37. Previously reported phytochemical contents in BBE might be the reason for antidiabetic activities. Quercetin was quantified at major content in BBE which is already reported for its antidiabetic biomarker role.

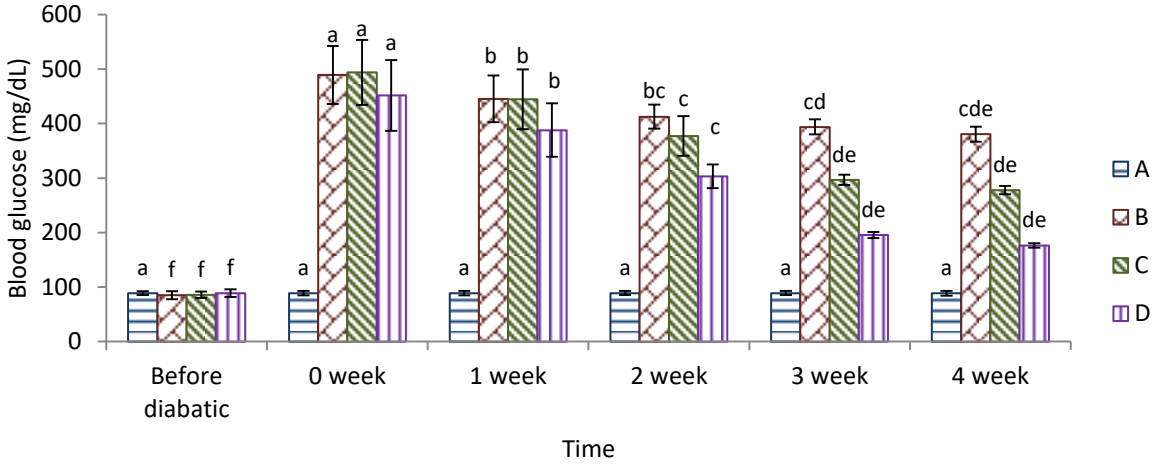

Fig. 7.3 Blood glucose of different treatment groups in A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated

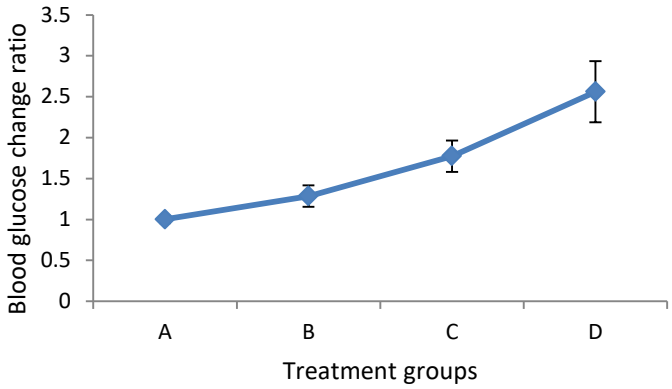

Fig. 7.4 Change in blood glucose of rats at different treatment groups, A-control, B-diabetic group no treatment, C- BBE treated, and D- Insulin treated

7.3.3.2 Effect of BBE on body weights of diabetic and non-diabetic rats

There was no unusual weight loss observed after oral dose of BBE on group of treatment rats (Fig. 7.5). A slight increment of body weights of rats were seen in the control group of rats (non-treated rats) up to 4th week in a normal way (Fig. 7.6). The significant weight loss was observed in group B-diabetic rats. Whereas there were no significant changes of weights were found in other group C (insulin treated diabetic rats) and group D (BBE treated diabetic rats). In DM-2, loss of body weight is common which is caused due to unable to glucose in sufficient way in adipose tissues [21]. In group D, insulin was already treating the diabetic conditions of rats, hence normalizing the excess weight loss up to 4th week of treatment. As BBE also should a great potential to treat diabetes in rats, hence there was no significant weight loss in group C (BBE treated diabetic rats), similarly as stated by Vilhena et al. [35].

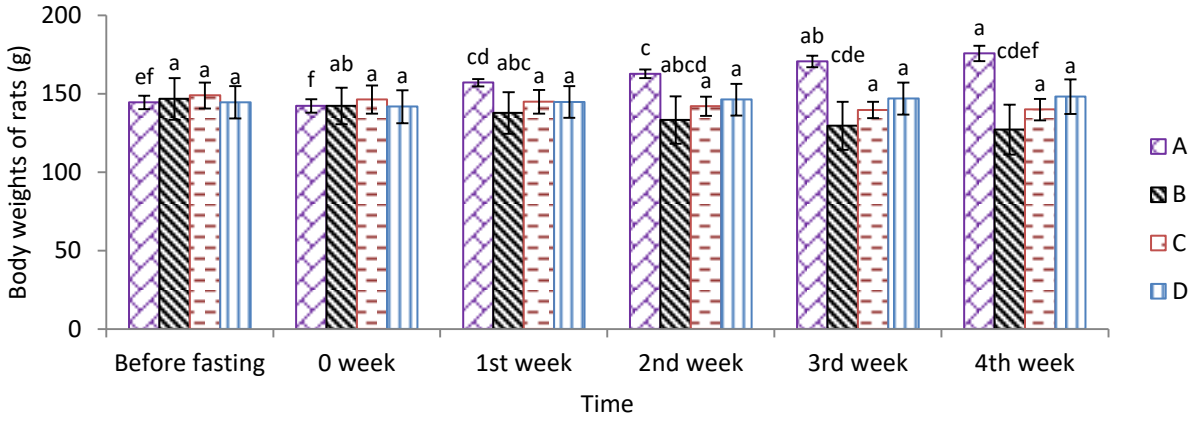

Fig. 7.5 Body weight of different treatment groups in A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated

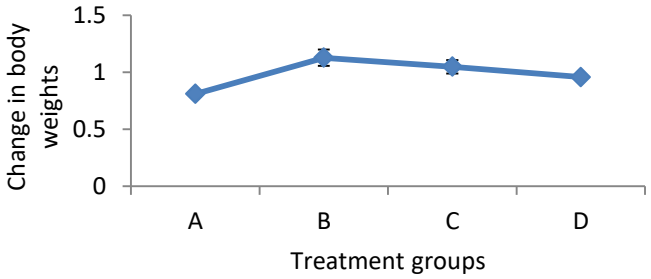

Fig. 7.6 Change in body weights of rats at different treatment groups, A-control, B-diabetic group no treatment, C- BBE treated, and D- Insulin treated

7.3.3.3 Glucose tolerance test (GTT) and insulin tolerance test (ITT)

The hierarchy of glucose tolerance over time was descending as the experimental group of rats, groups A>D>C>B (Fig. 7.7a). Group A, the control group (without treatment) of rats had the highest glucose tolerance level at the normal range of blood glucose. Moreover, group B (the diabetic group of rats) had the least glucose tolerance level and showed very high blood glucose levels after the oral dose of glucose. As some reports [6, 35], the diabetic rats treated with insulin (group D) and BBE (group C) showed significantly higher glucose tolerance than group B. The BBE treated diabetic rats (group C) showed blood glucose initially at 227.5±16.08 rose to 328±17.37 mg/dL at 30 min and dropped to 218.33±15.31 mg/dL at 120 min. Nevertheless, in the insulin tolerance test, blood glucose in experimental rat groups was seen to drop significantly initially to

30 min and then rose gradually till 120 min (Fig. 7.7b). The administration of insulin decreased the blood glucose significantly from group A<D<C<B. Moreover, in some diabetic conditions, insulin cannot be utilized by the body due to the dysfunction of insulin receptors caused due to damaged receptors. Due to the dysfunction of receptors, insulin cannot be utilized for blood regulation, which causes glucose accumulation in the bloodstream. In this study, insulin was given to each experimented group of rats to investigate insulin sensitivity and observe glucose deposition in the bloodstream. Investigation showed the BBE treated diabetic rats (group C) showed significantly high ($p \leq 0.05$) sensitive to insulin in contrary to diabetic rats (group B). This suggests that phytochemicals compound present in BBE have a strong ability to recover the dysfunctional insulin receptors and treat DM-1, indirectly similarly as stated by Ara et al. [6].

Overall high ability of BBE to increase the glucose tolerance level and significant insulin sensitivity in diabetic rats was the strong evidence justifying excellent antidiabetic properties of BBE at 500 mg/kg BW dose. As BBE contains a significant amount of phytochemicals (*viz*. phytate, chlorogenic acid, syringic acid, tannic acid, saponin, ferulic acid, caffeic acid, rutin, quercetin, catechin, gallic acid, sinapic acid, and coumarin) and other biochemical compounds which was previously reported with high antioxidant property [29]. According to many studies, many phytochemicals and bioactive compounds possess antioxidant and anti-inflammatory behaviors which have tendencies to recover cell damage and dysfunctions. Some cell damage is caused due to excessive lipids, some oxidative enzymes naturally present in the body, activation of endoplasmic stress pathway, and encourages to promote dysfunctions. Sometimes, insulin resistance is also caused due to pancreas. In this case, the pancreas produces excess insulin to regulate glucose in organs and cells, over time, insulin receptors are unable to use all the produced insulins. This result in receptors resistant to insulin and eventually, the pancreas keeps on producing more and more insulin to lower the blood glucose. This causes imbalance and dysfunction leading to a rise in blood glucose automatically [30].

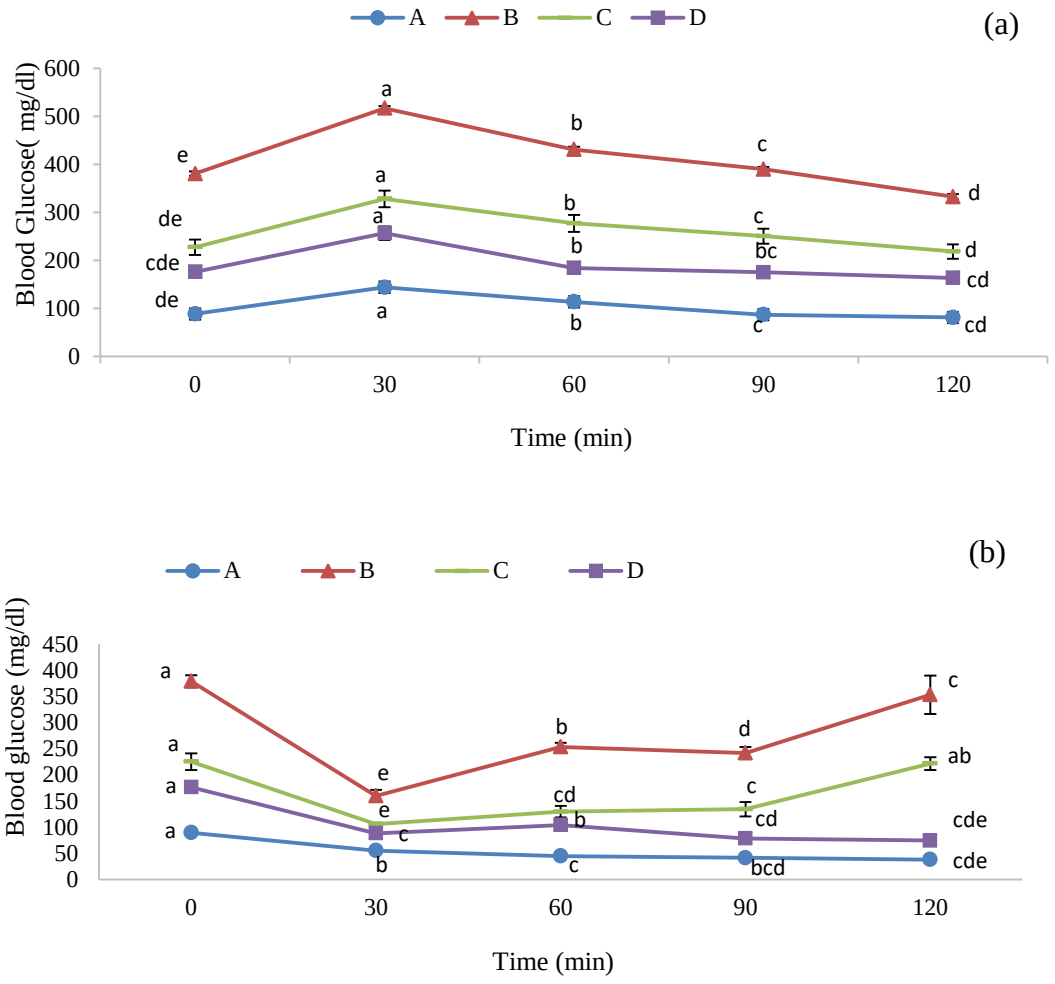

Fig. 7.7 Glucose tolerance level and insulin tolerance level of experimental group of rats; (a) Glucose tolerance test and (Insulin tolerance test), A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated

7.4 Conclusions

The investigation in this study showed a very positive outcome in treating DM. The phytochemical extract of bhimkol banana blossom was found to exhibit a strong ability to treat DM-1 and DM-2. Phytochemicals in BBE resulted in inhibiting some diabetic-enhancing enzymes (viz., α-amylase, α-glucosidase, and DPP-IV enzymes). The myoblast L6 cells were also found to uptake glucose effectively after the treatment with BBE. *In vivo* study of acute toxicity at 500 mg/kg BW of BBE concluded no toxic effects on Wister rats, hence it was then carried out for further investigation to treat diabetes in Wister rats. The study showed the very excellent ability of BBE to lower blood glucose

in diabetic rats without any unusual effect on their body weights. During the investigation of glucose tolerance level and insulin tolerance level, BBE enhanced the glucose tolerance level of BBE treated diabetic rats (group C) with increasing insulin sensitivity of insulin receptors, which might be due to the high antioxidant properties of BBE. All these positive results by the BBE might be due to its phytochemical compound presence (viz., quercetin, phytate, chlorogenic acid, syringic acid, tannic acid, saponin, ferulic acid, caffeic acid, rutin, catechin, gallic acid, sinapic acid, etc.) and other antioxidants. Therefore, this study suggests the application of BBE in food industries or the pharmaceutical sector to extract the benefits from this underutilized variety of banana blossom. Further investigation on the anti-cholesterolemic property of BBE is also encouraged positively.

References

[1] Akolade, J. O., Oloyede, H. O. B., and Onyenekwe, P. C. Encapsulation in chitosan-based polyelectrolyte complexes enhances antidiabetic activity of curcumin. *Journal of Functional Foods*, *35*, 584-594, 2017.

[2] Al-Masri, I. M., Mohammad, M. K., and Tahaa, M. O. Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*(5), 1061-1066, 2009.

[3] Alam, S., Sarker, M. M. R., Sultana, T. N., Chowdhury, M. N. R., Rashid, M. A., Chaity, N. I., Zhao, C., Xiao, J., Hafez, E. E., Khan, S. A., and Mohamed, I. N. Antidiabetic Phytochemicals From Medicinal Plants: Prospective Candidates for New Drug Discovery and Development. *Frontiers in Endocrinology*, *13*, 11, 2022.

[4] Alzorqi, I., Singh, A., Manickam, S., and Al-Qrimli, H. F. Optimization of ultrasound assisted extraction (UAE) of β- d -glucan polysaccharides from *Ganoderma lucidum* for prospective scale-up. *Resource-Efficient Technologies*, *3*(1), 46-54, 2017.

[5] Amalan, V., Vijayakumar, N., Indumathi, D., and Ramakrishnan, A. Antidiabetic and antihyperlipidemic activity of p-coumaric acid in diabetic rats, role of pancreatic GLUT 2: *In vivo* approach. *Biomedicine & Pharmacotherapy*, *84*, 230-236, 2016.

[6] Ara, F., Tripathy, A., and Ghosh, D. Possible Antidiabetic and Antioxidative Activity of Hydro-Methanolic Extract of *Musa balbisiana* (Colla) Flower in Streptozotocin-Induced Diabetic Male Albino Wistar Strain Rat: A Genomic Approach. *Assay and Drug Development Technologies*, *17*(2), 68-76, 2019.

[7] Ardalani, H., Hejazi Amiri, F., Hadipanah, A., and Kongstad, K. T. Potential antidiabetic phytochemicals in plant roots: a review of *in vivo* studies. *Journal of Diabetes and Metabolic Disorders*, *20*(2), 1837-1854, 2021.

[8] Bacanli, M., Dilsiz, S. A., Başaran, N., and Başaran, A. A. Effects of phytochemicals against diabetes. *Advances in Food and Nutrition Research*, *89*, 209-238, 2019.

[9] Barua, N. and Das, M. An overview on pharmacological activities of *Musa sapientum* and *Musa paradisiaca*. *International Journal of Pharma Professional's Research*, *4*(2), 852-858, 2013.

[10] Bhaskar, J. J., Kumar, A. P., and Salimath, P. V. Effect of banana (*Musa* sp. var. elakki bale) flower and pseudostem on antioxidant and lysosomal enzyme activities in streptozotocin-induced diabetic rats. *Journal of Pharmacy Research*, *4*(4), 1087-1091, 2011.

[11] Chen, S., Jiang, H., Wu, X., and Fang, J. Therapeutic Effects of Quercetin on Inflammation, Obesity, and Type 2 Diabetes. *Mediators of Inflammation*, *2016*, 2016.

[12] Choudhury, H., Pandey, M., Hua, C. K., Mun, C. S., Jing, J. K., Kong, L., Ern, L. Y., Ashraf, N. A., Kit, S. W., Yee, T. S., Pichika, M. R., Gorain, B, and Kesharwani, P. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. *Journal of Traditional and Complementary Medicine*, *8*(3), 361-376, 2018.

[13] Esteghamati, A., Larijani, B., Aghajani, M. H., Ghaemi, F., Kermanchi, J., Shahrami, A., Saadat, M., Esfahani, E. N., Ganji, M., Noshad, S., Khajeh, E., Ghajar, A., Heidari, B., Afarideh, M., Mechanick, J. I., and Ismail-Beigi, F. Diabetes in Iran: Prospective Analysis from First Nationwide Diabetes Report of National Program for Prevention and Control of Diabetes (NPPCD-2016). *Scientific Report*, *7*(1), 1-10, 2017.

[14] Etesami, B., Ghaseminezhad, S., Nowrouzi, A., Rashidipour, M., and RaziehYazdanparast. Investigation of 3T3-L1 Cell Differentiation to Adipocyte,

Affected by Aqueous Seed Extract of *Phoenix Dactylifera* L. *Reports of Biochemistry & Molecular Biology*, *9*(1), 14, 2020.

[15] Ganesan, K., Rana, M. B. M., and Sultan, S. (2022). Oral Hypoglycemic Medications. *StatPearls*. Retrieved August 08, 2022 from: https://www.ncbi.nlm.nih.gov/books/NBK482386/

[16] Gopalan, G., Prabha, B., Joe, A., Reshmitha, T. R., Sherin, D. R., Abraham, B., Sabu, M., Manojkumar, T. K., Radhakrishnana, K. V., and Nisha, P. Screening of *Musa balbisiana* Colla. seeds for antidiabetic properties and isolation of apiforol, a potential lead, with antidiabetic activity. *Journal of the Science of Food and Agriculture*, *99*(5), 2521-2529, 2019.

[17] Han, D. G., Cho, S. S., Kwak, J. H., and Yoon, I. S. Medicinal plants and phytochemicals for diabetes mellitus: pharmacokinetic characteristics and herb-drug interactions. *Journal of Pharmaceutical Investigation*, *49*(6), 603-612, 2019.

[18] Han, S., Middleton, P., Shepherd, E., Van Ryswyk, E., and Crowther, C. A. Different types of dietary advice for women with gestational diabetes mellitus. *Cochrane Database of Systematic Reviews*, *2017*(2), 2017.

[19] Huang, I., Lim, M. A., and Pranata, R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia - A systematic review, meta-analysis, and meta-regression. *Diabetes and Metabolic Syndrome*, *14*(4), 395, 2020.

[20] Kaleem, M., Asif, M., Ahmed, Q. U., and Bano, B. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. *Singapore Medical Journal*, *47*(8), 670, 2006.

[21] Kalita, H., Boruah, D. C., Deori, M., Hazarika, A., Sarma, R., Kumari, S., Kandimalla, R., Kotoki, J., and Devi, R. Antidiabetic and Antilipidemic Effect of *Musa balbisiana* Root Extract: A Potent Agent for Glucose Homeostasis in Streptozotocin-Induced Diabetic Rat. *Frontiers in Pharmacology*, *7*, 102, 2016.

[22] Kaul, K., Tarr, J. M., Ahmad, S., Kohner, E. M., and Chibber, R. Diabetes. In *Diabetes: An Old Disease, a New Insight*, pages 1–11. Springer, 2013.

[23] Liyanage, R., Gunasegaram, S., Visvanathan, R., Jayathilake, C., Weththasinghe, P., Chaminda Jayawardana, B., and Vidanarachchi, J. K. Experimental Diets Modulate Serum Cholesterol and Serum Glucose Level in Wistar Rats Fed with Cholesterol. *Cholesterol*, *2016*, 2016.

[24] Maksymchuk, O., Shysh, A., Rosohatska, I., and Chashchyn, M. Quercetin prevents type 1 diabetic liver damage through inhibition of CYP2E1. *Pharmacological Reports*, *69*(6), 1386-1392, 2017.

[25] Manikandan, R., Anand, A. V., and Kumar, S. Phytochemical and in vitro Antidiabetic activity of *Psidium Guajava* leaves. *Pharmacognosy Journal*, *8*(4), 2016.

[26] Marikkar, J. M. N., Tan, S. J., Salleh, A., Azrina, A., and Shukri, M. A. M. Evaluation of banana (*Musa* sp.) flowers of selected varieties for their antioxidative and anti-hyperglycemic potentials. *International Food Research Journal*, 23(5), 1988-1995, 2016.

[27] Mondal, I. H., Rangan, L., and Uppaluri, R. V. S. Parametric optimality of tray dried *Musa balbisiana* Colla blossom. *Journal of Food Science and Technology*, *57*(12), 4599-4612, 2020.

[28] Mozaffari-Khosravi, H., Afkhami-Ardekani, M., Fatehi, F., and Sadoughi, S. The effects of sour tea (*Hibiscus sabdariffa*) on hypertension in patients with type II diabetes. *Journal of Human Hypertension*, *23*(1), 48-54, 2009.

[29] Muchahary, S., and Deka, S. C. Impact of supercritical fluid extraction, ultrasound-assisted extraction, and conventional method on the phytochemicals and antioxidant activity of bhimkol (*Musa balbisiana*) banana blossom. *Journal of Food Processing and Preservation*, *45*(7), e15639, 2021.

[30] Muoio, D. M., and Newgard, C. B. Molecular and metabolic mechanisms of insulin resistance and β-cell failure in type 2 diabetes. *Nature Reviews Molecular Cell Biology*, *9*(3), 193–205, 2008.

[31] Nigatu, T. A., Afework, M., Urga, K., Ergete, W., and Makonnen, E. Toxicological investigation of acute and chronic treatment with *Gnidia stenophylla* Gilg root extract on some blood parameters and histopathology of spleen, liver and kidney in mice. *BMC Research Notes*, *10*(1), 625, 2017.

[32] Porwal, M., Khan, N. A., and Maheshwari, K. K. Evaluation of Acute and Subacute Oral Toxicity Induced by Ethanolic Extract of *Marsdenia tenacissima* Leaves in Experimental Rats. *Scientia Pharmaceutica*, *85*(3), 29, 2017.

[33] Rao, M. U., Sreenivasulu, M., Chengaiah, B., Reddy, K. J., and Chetty, C. M. Herbal Medicines for Diabetes Mellitus: A Review. *International Journal of PharmTech Research*, *2*(3), 1883-1892, 2010.

[34] Shi, G. J., Li, Y., Cao, Q. H., Wu, H. X., Tang, X. Y., Gao, X. H., Yu, J. Q., Chen, Z., and Yang, Y. *In vitro* and *in vivo* evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. *Biomedicine & Pharmacotherapy*, *109*, 1085-1099, 2019.

[35] Vilhena, R. O., Figueiredo, I. D., Baviera, A. M., Silva, D. B., Marson, B. M., Oliveira, J. A., Peccinini, R. G., Borges, I. K., and Pontarolo, R. Antidiabetic activity of *Musa* x *paradisiaca* extracts in streptozotocin-induced diabetic rats and chemical characterization by HPLC-DAD-MS. *Journal of Ethnopharmacology*, *254*, 112666, 2020.

[36] Vinué, Á., and González-Navarro, H. Glucose and insulin tolerance tests in the mouse. *Methods in Molecular Biology*, *1339*, 247–254, 2015.

[37] WHO. 2022. Diabetes. Retrieved October 6, 2022, from: https://www.who.int/health-topics/diabetes#tab=tab_1

[38] Zaman, M. M., Choudhury, S. R., Ahmed, J., Talukder, M. H., and Rahman, A. H. M. S. Blood glucose and cholesterol levels in adult population of Bangladesh: Results from STEPS 2006 survey. *Indian Heart Journal*, *68*(1), 52-56, 2016.

[39] Zhang, Y., Feng, F., Chen, T., Li, Z., and Shen, Q. W. Antidiabetic and antihyperlipidemic activities of *Forsythia suspensa* (Thunb.) Vahl (fruit) in streptozotocin-induced diabetes mice. *Journal of Ethnopharmacology*, *192*, 256-263, 2016.